



Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences

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Abstract

Approximately 500 species of ascomycetous yeasts, including members of *Candida* and other anamorphic genera, were analyzed for extent of divergence in the variable D1/D2 domain of large subunit (26S) ribosomal DNA. Divergence in this domain is generally sufficient to resolve individual species, resulting in the prediction that 55 currently recognized taxa are synonyms of earlier described species. Phylogenetic relationships among the ascomycetous yeasts were analyzed from D1/D2 sequence divergence. For comparison, the phylogeny of selected members of the *Saccharomyces* clade was determined from 18S rDNA sequences. Species relationships were highly concordant between the D1/D2 and 18S trees when branches were statistically well supported.

Introduction

Procedures commonly used for yeast identification rely on the appearance of cellular morphology and distinctive reactions on a standardized set of fermentation and assimilation tests. These tests are laborious and sometimes ambiguous because of strain variability. Given these difficulties and the impracticality of identifying most species from genetic crosses, molecular comparisons are increasingly used for yeast identification. Initial molecular work centered on determining the extent of nuclear DNA (nDNA) relatedness between isolates. Kurtzman (1987) and Kurtzman & Phaff (1987) reviewed results from nDNA reassociation studies of various heterothallic ascomycetous yeasts and noted that members of a biological species generally exhibit 70% or greater nDNA complementarity. Isolates with 40 to 70% nDNA relatedness are often considered varieties or subspecies unless genetic crosses indicate otherwise. These criteria have been applied to homothallic as well as to anamorphic (asexual) yeasts with the argument that strains of species from these groups appear to have neither greater nor

lesser ranges of nDNA relatedness than those found among heterothallic species.

Disadvantages of nDNA reassociation studies include the need for pairwise comparisons of all isolates under study and that resolution is limited to the genetic distance of sister species, i.e., closely related species that have only recently become genetically isolated from one another. As a result, interest has turned to other molecular comparisons that include sequencing, restriction fragment length polymorphisms (Bruns et al., 1991) and random amplified polymorphic DNA (Hadrys et al., 1992). Of these, sequencing appears the most robust because strain comparisons are easily made and, with the selection of appropriate genes, both close and distant relationships can be resolved. For example, Peterson & Kurtzman (1991) sequenced the variable D2 domain (ca. 300 nucleotides) near the 5' end of large subunit (26S) rRNA from selected heterothallic sister species in the genera *Issatchenkovia*, *Pichia* and *Saccharomyces* to determine if closely related species could be separated from substitutions in this region. Conspecific strains generally had fewer than 1% nucleotide substitutions in this domain, whereas biological species were separated by greater

than this number of substitutions, thus providing an empirical means for recognizing species.

Numerous studies have presented the phylogeny of different yeast groups from rRNA and rDNA sequence comparisons, but these studies have focused either on individual genera, which are usually circumscribed from phenotypic criteria, or on a relatively few widely divergent species. In either case, relationships are often incompletely understood because the number of taxa sampled has been small. To bring an overall perspective to species relationships, we compared sequences from the ca. 600-nucleotide D1/D2 domain (Guadet et al., 1989) at the 5' end of large subunit (LSU) rDNA for essentially all of the nearly 500 currently accepted species of ascomycetous yeasts, approximately 200 of which were included in our earlier studies (Kurtzman & Robnett, 1995; 1997). The data indicate that most yeast species can be identified from sequence divergence in the D1/D2 domain, and show that 55 currently accepted species are either synonyms or sister species of earlier described species. In addition, a phylogenetic analysis of the dataset provides an overview of close species relationships.

Materials and methods

Organisms

The strains studied are listed in Table 1, and all are maintained in the Agricultural Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research, Peoria, Illinois. Strains designated by a genus name followed by sp. are putative new species that will be described in future publications.

Growth of cultures, DNA isolation, PCR, and sequencing reactions

Cells used for DNA extraction were grown for approximately 24 h at 25 °C in 50 ml of Wickerham's (1951) YM broth (3 g yeast extract, 3 g malt extract, 5 g peptone, and 10 g glucose per liter of distilled water) on a rotary shaker at 200 rpm and harvested by centrifugation. The cells were washed once with distilled water, resuspended in 2 ml of distilled water, and 1 ml of the suspension was pipetted into each of two 1.5 ml microcentrifuge tubes. After centrifugation, excess water was decanted from the microcentrifuge tubes, and the packed cells were lyophilized for 1 to 2 days and stored in a freezer (-20 °C) until use.

DNA isolation for PCR was performed by a modified version of the sodium dodecyl sulfate protocol of Raeder & Broda (1985). The lyophilized cell mass from a single 1.5 ml microcentrifuge tube was broken apart with a pipette tip, and ca. 0.5 ml of 0.5-mm-diameter glass beads was added to the microcentrifuge tube. The tube was shaken for 20 min on a wrist action shaker at maximum speed. This treatment visibly fractured about 25% of the cells. The cells were suspended in 1 ml of extraction buffer (200 mM Tris-HCl [pH 8.5], 250 mM NaCl, 25 mM EDTA [pH 8.0], 0.5% sodium dodecyl sulfate) and extracted with phenol-chloroform and chloroform. As an alternative to the laboratory use of phenol, the broken cells were suspended in 700 µl 2X CTAB buffer (100 mM Tris-HCl [pH 8.4], 1.4 M NaCl, 25 mM EDTA, 2% hexadecyltrimethyl-ammonium bromide), vortex-mixed with an equal volume of chloroform and centrifuged for 10 min (K. O'Donnell pers. comm.). Following either extraction procedure, DNA was precipitated from the aqueous phase by adding 0.54 volume of isopropanol and pelleted for ca. 3 min in an Eppendorf model 5415 microcentrifuge at 14,000 rpm. The pellet was washed gently with 70% ethanol, resuspended in 100 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]), and dissolved by incubation at 55 °C for 1 to 2 h. Dilute DNA samples for PCR were prepared by adding 4 µl of the genomic stocks to 1 ml of 0.1X TE buffer.

The divergent D1/D2 domain (nucleotides 63–642 for *Saccharomyces cerevisiae*) at the 5' end of the LSU rRNA gene was symmetrically amplified with primers NL-1 (5'-GCATATCAATAAGCGGAGGAAAG) and NL-4 (5'-GGTCCGTGTTCAAGACGG) (O'Donnell, 1993). Amplification was performed for 36 PCR cycles with annealing at 52 °C, extension at 72 °C for 2 min, and denaturation at 94 °C for 1 min. The amplified DNA was purified with Geneclean II (Bio 101, La Jolla, Calif.) according to the manufacturer's instructions. Visualization of the amplified DNA was performed following Geneclean II treatment by electrophoresis in 1.5% agarose in 1X TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA [pH 8.0]) and staining with ethidium bromide (8×10^{-5} µg/µl).

Both strands of the rDNA regions compared were sequenced with the ABI TaqDyeDeoxy Terminator Cycle sequencing kit (Applied Biosystems Inc., Foster City, Calif.). Four sequencing reactions were performed for each DNA sample. Primers for these reactions were the external primers NL-1 and NL-4 and the internal primers NL-2A

Table 1. Strains of ascomycetous yeasts and reference species compared

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>Aciculonidium aculeatum</i>	YB-4298 ^T	5578		U40087
	YB-4297	5293	2	
<i>Ambrosiozyma (Hormoascus) ambrosiae</i>	Y-7524 ^T	6003		U73605
<i>A. cicatricosa</i>	Y-17594 ^T	6157		U40128
<i>A. monospora</i>	Y-1484 ^T	2554		U40106
<i>A. (Hormoascus) philentoma</i>	Y-7523 ^T	6276		U40113
<i>A. (Hormoascus) platypodis</i>	Y-6732 ^T	4111		U40083
<i>Arxiozyma telluris</i>	YB-4302 ^T	2685		U72158
<i>Arxula adeninivorans</i>	Y-17692 ^T	8244		U40094
	Y-17693	7370	2	
	Y-17851	6461	2	
	Y-17993		2	
<i>A. terrestris</i>	Y-17704 ^T	7376		U40103
<i>Ascoidea africana</i>	Y-17632 ^T	377.68		U40131
<i>A. corymbosa</i>	Y-17576 ^T	457.69		
<i>A. hylecoeti</i>	Y-17634 ^A	355.80		U76198
	Y-17703		0	
<i>A. rubescens</i>	Y-17699 ^A	116.35		U76195
	Y-17700	111.48	0	
	Y-17702		0	
<i>Blastobotrys arbuscula</i>	Y-17585 ^T	227.83		U40108
<i>B. aristata</i>	Y-17579 ^T	521.75		U40109
<i>B. capitulata</i>	Y-17573 ^T	287.82		U40104
<i>B. elegans</i>	Y-17572 ^T	530.83		U40095
<i>B. nivea</i>	Y-17581 ^T	163.67		U40110
<i>B. proliferans</i>	Y-17577 ^T	522.75		U40098
<i>Blastobotrys</i> sp.	YB-2092			AF017247
<i>Botryozyma nematodophila</i>	Y-17705 ^T	7426		U40105
	Y-17706	7442	0	
<i>Brettanomyces custersianus</i>	Y-6653 ^T	4805		U76199
<i>B. naardenensis</i>	Y-17526 ^T	6042		U76200
<i>B. (Eeniella) nanus</i>	Y-17527 ^T	1945		U76197
<i>Candida aaseri</i>	YB-3897 ^T	1913		U45802
	Y-6955		0	
<i>C. agrestis</i>	Y-17640 ^T	8055		U71074
<i>C. albicans</i>	Y-12983 ^{NT}	562		U45776
	Y-17967		1	
	Y-17968		1	
	Y-17974		1	
	Y-17976		2	
	YB-3898	1912	1	
<i>C. amapae</i>	Y-17845 ^T	7872		U69880
<i>C. anatomiae</i>	Y-17641 ^T	5547		U70244
<i>C. ancudensis</i>	Y-17327 ^T	8184		U45810
<i>C. antillancae</i>	Y-17673 ^T	8170		U45812
<i>C. apicola</i>	Y-2481 ^T	2868		U45703

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>C. apis</i>	Y-2482 ^T	2674		U48237
<i>C. atlantica</i>	Y-17759 ^T	5263		U45799
<i>C. atmosphaerica</i>	Y-17642 ^T	4547		U45779
<i>C. auringiensis</i>	Y-17674 ^T	6913		U62300
<i>C. austromarina</i>	Y-17769 ^T	6179		U62310
	Y-17852	6588	0	
<i>C. azyma</i>	Y-17067 ^T	6826		U62312
<i>C. beecheii</i>	Y-17758 ^T	4261		U45798
<i>C. bertae</i> var. <i>bertae</i>	Y-17643 ^T	8169		U70251
<i>C. bertae</i> var. <i>chiloensis</i>	Y-17646 ^T	8168		AF017236
<i>C. berthetii</i>	Y-17644 ^T	5452		U62298
<i>C. blankii</i>	Y-17068 ^T	1898		U45704
<i>C. boidinii</i>	Y-2332 ^T	2428		U70242
	Y-17325	8030	0	
<i>C. boleticola</i>	Y-17080 ^T	6420		U45777
<i>C. bombi</i>	Y-17081 ^T	5836		U45706
<i>C. bombicola</i>	Y-17069 ^T	6009		U45705
<i>C. bondarzewiae</i>	Y-17328 ^T	8171		U45806
<i>C. buinensis</i>	Y-11706 ^T	6796		U45778
<i>C. butyri</i>	Y-17648 ^T	6421		U45780
<i>C. cacaoi</i>	Y-17649 ^T	2020		U45744
<i>C. cantarellii</i>	Y-17650 ^T	4878		U45814
<i>C. cariosilignicola</i>	Y-11996 ^T	8001		U70188
<i>C. caseinolytica</i>	Y-17796 ^T	7881		U70250
<i>C. castellii</i>	Y-17070 ^T	4332		U69876
<i>C. castrensis</i>	Y-17329 ^T	8172		U45807
<i>C. catenulata</i>	Y-1508 ^T	565		U45714
<i>C. cellulolytica</i>	Y-17856 ^T	7920		U94928
<i>C. chilensis</i>	Y-7790 ^T	5719		U45821
<i>C. chiropterorum</i>	Y-17071 ^T	6064		U45822
<i>C. coipomoensis</i>	Y-17651 ^T	8178		U45747
<i>C. conglobata</i>	Y-1504 ^T	2018		U45789
<i>C. cylindracea</i>	Y-17506 ^T	6330		U45823
<i>C. dendrica</i>	Y-7775 ^T	6151		U62301
<i>C. dendronema</i>	Y-7781 ^T	6270		U45751
<i>C. diddensiae</i>	Y-7589 ^T	2214		U45750
	YB-4907		0	
<i>C. diversa</i>	Y-5713 ^T	4074		U71064
<i>C. drimydis</i>	Y-17675 ^T	8185		U45815
<i>C. dubliniensis</i>	Y-17841 ^T	7987		U57685
	Y-17512		0	
	Y-17969		0	
	Y-17971		0	
	Y-17972		0	
	Y-17973		0	
	Y-17975		1	

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>C. edax</i>	Y-17083 ^T	5657		U70245
<i>C. entomaea</i>	Y-7785 ^T	6306		U45790
<i>C. entomophila</i>	Y-7783 ^T	6160		U62302
<i>C. ergastensis</i>	Y-17652 ^T	6248		U45746
<i>C. ernobii</i>	Y-17782 ^T	1737		U70241
	Y-12941	4863	0	
<i>C. etchellsii</i>	Y-17084 ^T	1750		U45723
	YB-4333		0	
<i>C. ethanolica</i>	Y-12615 ^T	8041		U71073
<i>C. fennica</i>	Y-7505 ^T	5928		U45715
<i>C. fermenticarens</i>	Y-17321 ^T	7040		U45756
<i>C. floricola</i>	Y-17676 ^T	7289		U45710
<i>C. fluviatilis</i>	Y-7711 ^T	6776		U45717
<i>C. fragi</i>	Y-17910 ^T	7702		U71071
<i>C. freyschussii</i>	Y-7957 ^A	2161		AF017242
<i>C. friedrichii</i>	Y-17653 ^T	4114		U45781
<i>C. fructus</i>	Y-17072 ^T	6380		U44810
<i>C. fukuyamaensis</i>	Y-17857 ^T	7921		U62311
<i>C. galacta</i>	Y-17645 ^T	6939		U45820
<i>C. geochares</i>	Y-17073 ^T	6870		U48591
<i>C. glabrata</i>	Y-65 ^T	138		U44808
	Y-17815		1	
<i>C. glaeiosa</i>	Y-6949 ^T	5691		U45757
<i>C. glucosiphila</i>	Y-17781 ^T	7349		U45849
<i>C. gropengiesseri</i>	Y-1445 ^T	156		U45721
<i>C. haemulonii</i>	Y-6693 ^T	5149		U44812
	Y-17799	5150	0	
	Y-17800	7801	0	
<i>C. haemulonii</i> Type II	Y-17801	6915		U44819
	Y-17802	7798	0	
<i>C. halonitratophila</i>	Y-6694 ^T	5240		U45725
<i>C. halophila</i>	Y-2483 ^T	4019		U45828
<i>C. homilentoma</i>	Y-10941 ^T	6312		U45716
<i>C. humilis</i>	Y-17074 ^T	5658		U69878
<i>C. incommunis</i>	Y-17085 ^T	5604		U62303
<i>C. inconspicua</i>	Y-2029 ^T	180		U71062
<i>C. insectalens</i>	Y-7778 ^T	6036		U62304
<i>C. insectamans</i>	Y-7786 ^T	6033		U45753
<i>C. insectorum</i>	Y-7787 ^T	6213		U45791
<i>C. intermedia</i>	Y-981 ^T	572		U44809
<i>C. ishiwadae</i>	Y-17654 ^T	6022		U71067
<i>C. karawaiewii</i>	Y-17784 ^T	5214		U94921
<i>C. krissii</i>	Y-17086 ^T	6519		U45853
<i>C. kruisii</i>	Y-17087 ^T	6451		U45718
<i>C. lactis-condensi</i>	Y-1515 ^T	52		U45724
	Y-2338	2633	0	

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>C. laureliae</i>	Y-17656 ^T	8180		U45787
<i>C. llanquihuensis</i>	Y-17657 ^T	8182		U70190
<i>C. lodderae</i>	Y-17317 ^T	1924		U45755
<i>C. lyxosopha</i>	Y-17539 ^T	8194		U76204
	Y-17538	7268	0	U45801
<i>C. magnifica</i> ^d	Y-5717			U44821
<i>C. magnoliae</i>	Y-2024 ^T	166		U45722
<i>C. maltosa</i>	Y-17677 ^T	5611		U45745
<i>C. mannitofaciens</i>	Y-7226 ^T	5981		U45830
<i>C. maris</i>	Y-6696 ^T	5151		U70181
<i>C. maritima</i>	Y-17775 ^T	5107		U69877
<i>C. melibiosica</i>	Y-17076 ^T	5814		U44813
<i>C. membranifaciens</i>	Y-2089 ^T	1952		U45792
<i>C. mesenterica</i>	Y-1494 ^T	602		U45720
<i>C. methanolophaga</i>	Y-17658 ^T	7297		U94925
<i>C. methanosorbosa</i>	Y-17320 ^T	7029		U70186
<i>C. milleri</i>	Y-7245 ^T	6897		U94923
<i>C. mogii</i>	Y-17032 ^T	2032		U44820
<i>C. molischiana</i>	Y-2237 ^T	136		U70178
<i>C. montana</i>	Y-17326 ^T	8057		U62305
<i>C. multigemmis</i>	Y-17659 ^T	6524		U45782
<i>C. musae</i>	Y-17088 ^T	6381		U44814
<i>C. naeodendra</i>	Y-10942 ^T	6032		U45759
<i>C. nanaspora</i>	Y-17679 ^T	7200		U70187
<i>C. natalensis</i>	Y-17680 ^T	2935		U45818
<i>C. nemodendra</i>	Y-7779 ^T	6280		U70246
<i>C. nitratophila</i>	YB-3654 ^T	2027		U70180
	Y-5872		0	
<i>C. nodaensis</i>	Y-2484 ^T	3094		U45726
<i>C. norvegica</i>	Y-17660 ^T	4239		U62299
	YB-3946		1	
	YB-3949		1	
	YB-3950		0	
<i>C. odintsovae</i>	Y-17760 ^T	6026		U70182
<i>C. oleophila</i>	Y-2317 ^T	2219		U45793
<i>C. ootensis</i>	Y-17661 ^T	7299		U94926
<i>C. oregonensis</i>	Y-5850 ^T	5036		U44815
	YB-4493		0	
<i>C. ovalis</i>	Y-17662 ^T	7298		U70248
<i>C. palmioleophila</i>	Y-17323 ^T	7418		U45758
	Y-17834		0	
<i>C. paludigena</i>	Y-12697 ^T	8005		U45826
<i>C. parapsilosis</i>	Y-12969 ^T	604		U45754
	Y-543	2197	0	
<i>C. pararugosa</i>	Y-17089 ^T	1010		U62306
	Y-7031		2	

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>C. peltata</i>	Y-6888 ^T	5576		U71066
<i>C. petrohuensis</i>	Y-17663 ^T	8173		U45819
<i>C. pignaliae</i>	Y-17664 ^T	6071		U70183
<i>C. pini</i>	Y-2023 ^T	970		U70252
<i>C. populi</i>	Y-17681 ^T	7351		U70249
	Y-27040	7352	0	
<i>C. pseudoglaebosa</i>	Y-17911 ^T	6715		U71072
<i>C. pseudointermedia</i>	Y-10939 ^T	6918		U44816
<i>C. pseudolambica</i>	Y-17318 ^T	2063		U71063
<i>C. psychrophila</i>	Y-17665 ^T	5956		U45813
<i>C. quercitrusa</i>	Y-5392 ^T	4412		U45831
	Y-5704		1	
<i>C. quercuum</i>	Y-12942 ^T	6422		U70184
<i>C. railenensis</i>	Y-17762 ^T	8164		U45800
<i>C. ralunensis</i>	Y-17666 ^T	8179		U45786
<i>C. rhagii</i>	Y-2594 ^T	4237		U45729
<i>C. rugopelliculosa</i>	Y-17079 ^T	6377		U71069
<i>C. rugosa</i>	Y-95 ^T	613		U45727
<i>C. saitoana</i>	Y-17316 ^T	940		U45762
<i>C. sake</i>	Y-1622 ^T	159		U45728
	Y-1499	617	0	
<i>C. salmanticensis</i>	Y-17090 ^T	5121		U62308
<i>C. santamariae</i> var. <i>santamariae</i>	Y-6656 ^T	4515		U45794
<i>C. santamariae</i> var. <i>membranifaciens</i>	Y-17647 ^T	5838		U45785
<i>C. santjacobensis</i>	Y-17667 ^T	8183		U45811
<i>C. savonica</i>	Y-17077 ^T	6563		U62307
<i>C. schatavii</i>	Y-17078 ^T	6452		U45795
<i>C. sequanensis</i>	Y-17682 ^T	8118		U45711
<i>C. shehatae</i> var. <i>shehatae</i>	Y-12858 ^T	5813		U45761
<i>C. shehatae</i> var. <i>insectosa</i>	Y-12854 ^T	4286		U45773
<i>C. shehatae</i> var. <i>lignosa</i>	Y-12856 ^T	4705		U45772
<i>C. silvae</i>	Y-6725 ^T	5498		U71065
<i>C. silvanorum</i>	Y-7782 ^T	6274		U71068
<i>C. silvatica</i>	Y-7777 ^T	6277		U76201
<i>C. silvicultrix</i>	Y-7789 ^T	6269		U69879
<i>C. sojae</i>	Y-17909 ^T	7871		U71070
<i>C. solani</i>	Y-2224 ^T	1908		U70179
<i>C. sonorensis</i>	Y-7800 ^T	6792		U70185
<i>C. sophiae-reginae</i>	Y-17668 ^T	8175		U45817
<i>C. sorbophila</i>	Y-7921 ^T	6739		U45852
<i>C. sorboxylosa</i>	Y-17669 ^T	6378		U62314

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>C. spandovensis</i>	Y-17761 ^T	6875		U62309
<i>C. stellata</i>	Y-1446 ^T	157		U45730
<i>C. stellimalicola</i>	Y-17912 ^T	7853		U84234
<i>C. succiphila</i>	Y-11998 ^T	8003		U70189
<i>C. suecica</i>	Y-12943 ^T	5724		U45732
<i>C. tanzawaensis</i>	Y-17324 ^T	7422		U44811
<i>C. tenuis</i>	Y-1498 ^T	615		U45774
<i>C. tepae</i>	Y-17670 ^T	5115		U45816
<i>C. terebra</i>	Y-17683 ^T	6023		U45784
<i>C. torresii</i>	Y-6699 ^T	5152		U45731
<i>C. tropicalis</i>	Y-12968 ^T	94		U45749
	Y-5716	4913	0	
	Y-1552	433	0	
<i>C. tsuchiyae</i>	Y-17840 ^T	7195		U49064
<i>C. vaccinii</i>	Y-17684 ^T	7318		U45708
<i>C. valdiviana</i>	Y-7791 ^T	5721		U45835
<i>C. vanderwaltii</i>	Y-17671 ^T	5524		U62313
<i>C. vartiovaarae</i>	Y-6701 ^T	4289		U69875
<i>C. veronae</i>	Y-17672 ^T	5815		U45783
<i>C. versatilis</i>	Y-6652 ^T	1752		U45834
<i>C. vinaria</i>	Y-5715 ^T	4077		U45833
<i>C. vini</i>	Y-1615 ^{NT}	639		
	Y-1616	640	0	
	Y-17853	634	2	
	Y-17854	639	0	
<i>C. viswanathii</i>	Y-6660 ^T	4024		U45752
<i>C. wickerhamii</i>	Y-2563 ^T	2928		U70243
<i>C. xestobii</i>	Y-17685 ^T	5975		U45707
<i>C. zeylanoides</i>	Y-1774 ^T	619		U45832
<i>Candida</i> sp.	Y-6843			AF017241
<i>Candida</i> sp.	Y-6487			
	Y-6488		0	AF017240
<i>Candida</i> sp.	Y-8257			AF017243
<i>Candida</i> sp.	Y-17456			U45775
<i>Candida</i> sp.	Y-17713			AF017238
<i>Candida</i> sp.	Y-17858	7922		AF017239
<i>Candida</i> sp.	YB-1246			AF017244
<i>Candida</i> sp.	YB-1272			AF017245
<i>Candida</i> sp.	YB-2248			AF017248
<i>Candida</i> sp.	YB-2694			AF017249
<i>Candida</i> sp.	YB-3657			AF017726
<i>Cephaloascus albidus</i>	Y-7343 ^T	389.77		U39474
<i>C. fragrans</i>	Y-6742 ^T	121.29		U40091
<i>Citeromyces matritensis</i>	Y-2407 ^T	2764		U75959
<i>Clavispora lusitaniae</i>	Y-11827 ^T	6936		U44817
<i>C. opuntiae</i>	Y-11820 ^T	7068		U44818

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>Cyniclomyces guttulatus</i>	Y-17561 ^A			U76196
<i>Debaryomyces (Pichia) carsonii</i>	YB-4275 ^T	2285		U45743
<i>D. castellii</i>	Y-7423 ^T	2923		U45841
<i>D. coudertii</i>	Y-7425 ^T	5167		U45846
<i>D. (Pichia) etchellsii</i>	Y-7121 ^T	2011		U45809
<i>D. halotolerans</i>	Y-7478 ^T	5949		
<i>D. hansenii</i> var. <i>hansenii</i>	Y-7426 ^T	767		U45808
	YB-150		0	
	YB-1221		0	
	YB-3617		0	
<i>D. hansenii</i> var. <i>fabryi</i>	Y-17914 ^T	789		U94927
<i>D. maramus</i>	Y-2171 ^T	1958		U45838
<i>D. melissophilus</i>	Y-7585 ^T	6344		U45740
<i>D. nepalensis</i>	Y-7108 ^T	5921		U45839
<i>D. (Schwanniomyces) occidentalis</i> var. <i>occidentalis</i>	Y-10 ^T	819		U45804
<i>D. (Schwanniomyces) occidentalis</i> var. <i>persoonii</i>	Y-7400 ^T	2169		U45840
<i>D. polymorphus</i>	Y-2022 ^T	186		U45836
	Y-7413		0	
<i>D. pseudopolymorphus</i>	YB-4229 ^T	2008		U45845
<i>D. (Wingea) robertsiae</i>	Y-6670 ^T	2934		U45805
<i>D. tamarii</i>	Y-6665 ^T	4333		U94920
<i>D. udenii</i>	Y-17354 ^T	7056		U45844
<i>D. vanrijiae</i> var. <i>vanrijiae</i>	Y-7430 ^T	3024		U45842
<i>D. vanrijiae</i> var. <i>yarrowii</i>	Y-7535 ^T	6246		U45843
<i>D. yamadae</i>	Y-11714 ^T	7035		U45837
<i>Debaryomyces</i> sp.	Y-7804			U45771
<i>Dekkera anomala</i>	Y-17522 ^T	8139		U84244
	Y-1415	77	1	
<i>D. bruxellensis</i>	Y-12961 ^T	74		U45738
<i>Dipodascopsis tothii</i>	Y-12690 ^T	759.85		U40101
<i>D. uninucleata</i> var. <i>uninucleata</i>	Y-17583 ^T	190.37		U40137
<i>D. uninucleata</i> var. <i>wickerhamii</i>	Y-2181 ^T	741.74		U40136
<i>Dipodascus aggregatus</i>	Y-17564 ^T	175.53		U40120
<i>D. albidus</i>	Y-12859 ^A	766.85		U40081
<i>D. ambrosiae</i>	Y-17575 ^T	749.85		U40102
<i>D. armillariae</i>	Y-17580 ^T	817.71		U40093
<i>D. australiensis</i>	Y-17565 ^T	625.74		U40100
<i>D. capitatus</i>	Y-17686 ^T	197.35		U40084
<i>D. geniculatus</i>	Y-17628 ^T	184.80		U40130
<i>D. ingens</i>	Y-17630 ^A	521.90		U40127
	Y-10929	6787	3	U40134
<i>D. macrosporus</i>	Y-17586 ^T	259.82		U40121
<i>D. magnusii</i>	Y-17563 ^A	108.12		U40097
<i>D. ovetensis</i>	Y-17574 ^T	192.55		U40116
<i>D. spicifer</i>	Y-17578 ^T	244.85		U40115

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>D. starmeri</i>	Y-17816 ^T	780.96		U59391
	Y-17817	781.96	0	
<i>D. tetrasperma</i>	Y-7288 ^T	765.70		U40086
	YB-1854		1	
' <i>Endomyces decipiens</i> '	Y-17609	165.29		U40129
<i>Eremothecium ashbyi</i>	Y-1363 ^A			U43387
	Y-7249		1	
<i>E. (Nematospora) coryli</i>	Y-12970 ^T	2608		U43390
	Y-1618	2599	0	
	Y-7133		2	
<i>E. cymbalariae</i>	Y-17582 ^A	270.75		U43388
<i>E. (Ashbya) gossypii</i>	Y-1056 ^A	109.51		U43389
	Y-1810		0	
<i>E. (Holleya) sinecaudum</i>	Y-17231 ^T	8199		U43391
<i>Galactomyces citri-aurantii</i>	Y-17913 ^T	175.89		U84233
	Y-17923	176.89	0	
<i>G. geotrichum</i>	Y-17569 ^T	772.71		U40118
<i>G. reessii</i>	Y-17566 ^T	179.60		U40111
<i>Galactomyces</i> sp.	Y-6418			AF017396
<i>Geotrichum clavatum</i>	Y-17570 ^A	425.71		U40112
<i>G. fermentans</i>	Y-17567 ^T	439.83		U40117
<i>G. fragrans</i>	Y-17571 ^A	152.25		U40119
<i>G. klebahnii</i>	Y-17568 ^A	179.30		U40114
<i>Geotrichum</i> sp.	Y-5419			AF017397
<i>Hanseniaspora guilliermondii</i>	Y-1625 ^T	465		U84230
<i>H. (Kloeckeraspora) occidentalis</i>	Y-7946 ^T	2592		U84225
	YB-4040		0	
<i>H. (Kloeckeraspora) osmophila</i>	Y-1613 ^T	313		U84228
<i>H. uvarum</i>	Y-1614 ^T	314		U84229
<i>H. valbyensis</i>	Y-1626 ^T	479		U73596
<i>H. (Kloeckeraspora) vineae</i>	Y-17529 ^T	2171		U84224
' <i>Hansenula arabitolgenes</i> '	Y-17244 ^A	7164		
' <i>H. misumaiensis</i> '	Y-17389 ^A	8062		U73581
	YB-3520		0	
<i>Issatchenka occidentalis</i>	Y-7552 ^T	5459		U76348
	Y-6545		0	
	Y-7767	1910	0	
	YB-3389		0	
<i>I. orientalis</i>	Y-5396 ^T	5147		U76347
	Y-7550	2911	0	
	Y-7724	5590	0	
<i>I. scutulata</i> var. <i>scutulata</i>	Y-7663 ^T	6670		U76529
	Y-11602		1	
<i>I. scutulata</i> var. <i>exigua</i>	Y-10920 ^T	6836		U76349
	Y-11604		3	
<i>I. terricola</i>	YB-4310 ^T	2617		U76345

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>Issatchenkia</i> sp.	Y-8218		0	
<i>Issatchenkia</i> sp.	Y-12824			AF017398
	Y-12825		0	
<i>Issatchenkia</i> sp.	Y-12827			AF017235
<i>Issatchenkia</i> sp.	Y-12830			AF017399
<i>Kloeckera lindneri</i>	Y-17531 ^T	285		U84226
<i>Kluuyveromyces aestuarii</i>	YB-4510 ^T	4438		U69579
<i>K. africanus</i>	Y-8276 ^T	2517		U68550
<i>K. bacillisporus</i>	Y-17846 ^T	7720		U69583
<i>K. blattae</i>	Y-10934 ^T	6284		U69580
<i>K. delphensis</i>	Y-2379 ^T	2170		U69576
<i>K. dozhanskii</i>	Y-1974 ^T	2104		U69575
<i>K. lactis</i> var. <i>lactis</i>	Y-8279 ^T	683		U94922
<i>K. lactis</i> var. <i>drosophilicola</i>	Y-8278 ^T	2105	0	U94919
<i>K. lodderae</i>	Y-8280 ^T	2757		U68551
<i>K. marxianus</i>	Y-8281 ^T	712		U94924
	Y-2415	397	0	
<i>K. phaffii</i>	Y-8282 ^T	4417		U69578
<i>K. piceae</i>	Y-17977 ^T	7738		U84346
<i>K. polysporus</i>	Y-8283 ^T	2163		U68548
<i>K. thermotolerans</i>	Y-8284 ^T	6340		U69581
<i>K. waltii</i>	Y-8285 ^T	6430		U69582
<i>K. wickerhamii</i>	Y-8286 ^T	2745		U69577
	Y-17733		0	
<i>K. yarrowii</i>	Y-17763 ^T	8242		U68559
<i>Lipomyces (Babjevia) anomalus</i>	Y-7931 ^T	6740		U76202
<i>L. (Smithiozyma) japonicus</i>	Y-17848 ^T	7319		U94947
<i>L. kononenkoae</i> subsp. <i>kononenkoae</i>	Y-11553 ^T	2514		U84235
<i>L. kononenkoae</i> subsp. <i>spencer-martinsiae</i>	Y-7042 ^T	5608		U84237
<i>L. (Waltomyces) lipofer</i>	Y-11555 ^T	944		U76533
<i>L. starkeyi</i>	Y-11557 ^T	1807		U45824
<i>L. tetrasporus</i>	Y-11562 ^T	5910		U76527
<i>Lodderomyces elongisporus</i>	YB-4239 ^T	2605		U45763
	Y-7681	5912	0	
<i>Mastigomyces philippovii</i>	Y-17708 ^{NT}	7047		U45760
<i>Metschnikowia agaves</i>	Y-17915 ^T	7744		U84243
	Y-27041	7745	5	
<i>M. australis</i>	Y-17414 ^T	5847		U76526
	Y-7014	5848	0	U44824
<i>M. bicuspidata</i> var. <i>bicuspidata</i>	YB-4993 ^{NT}	5575		U44822
<i>M. bicuspidata</i> var. <i>californica</i>	Y-17916 ^T	6010		U94944
<i>M. bicuspidata</i> var. <i>chathamia</i>	Y-17917 ^T	5980		U84238
<i>M. gruessii</i>	Y-17809 ^T	7657		U45737
<i>M. hawaiiensis</i>	Y-17272 ^T	7432		U45734
<i>M. krissii</i>	Y-5389 ^T	4823		U45735
<i>M. lunata</i>	Y-7131 ^T	5946		U45733

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>M. pulcherrima</i>	Y-7111 ^T	5833		U45736
<i>M. reukaufii</i>	Y-7112 ^T	5834		U44825
<i>M. zobellii</i>	Y-5387 ^T	4821		U44823
<i>Metschnikowia</i> sp.	Y-5941			AF017400
<i>Metschnikowia</i> sp.	Y-6148	5536		AF017401
<i>Metschnikowia</i> sp.	Y-6344			AF017402
<i>Metschnikowia</i> sp.	Y-7275			AF017403
<i>Metschnikowia</i> sp.	Y-17036			AF017404
<i>Myxozyma geophilica</i>	Y-17252 ^T	7219		U76351
	Y-17246	7037	0	
<i>M. kluuyveri</i>	Y-17277 ^T	7332		U76352
<i>M. lipomycoidea</i>	Y-17253 ^T	7038		U76350
<i>M. melibiosi</i>	Y-11781 ^T	2102		U76344
<i>M. monticola</i>	Y-17726 ^T	7806		U76355
<i>M. mucilagina</i>	Y-11823 ^T	7071		U94945
<i>M. neotropica</i>	Y-17859 ^A	7953		U76356
<i>M. udenii</i>	Y-17387 ^T	7439		U76353
<i>M. vanderwaltii</i>	Y-17727 ^T	7793		U76354
<i>Nadsonia commutata</i>	Y-7950 ^T	6640		U73598
<i>N. fulvescens</i> var. <i>fulvescens</i>	Y-12810 ^T	2596		U45825
<i>N. fulvescens</i> var. <i>elongata</i>	Y-1568 ^T	2594		U94942
	Y-991		0	
	Y-1617	2593	0	
<i>Pachysolen tannophilus</i>	Y-2460 ^T	4044		U76346
<i>Pichia abadieae</i>	Y-7499 ^T	6067		U46123
<i>P. (Yamadazyma) acaciae</i>	Y-7117 ^T	5656		U45767
	Y-7773	5985	0	
<i>P. alni</i>	Y-11625 ^T	6986		U74596
<i>P. americana</i>	Y-2156 ^T	5644		U73575
<i>P. amethionina</i> var. <i>amethionina</i>	Y-10978 ^T	6940		U75424
<i>P. amethionina</i> var. <i>pachycereana</i>	Y-10981 ^T	6943		U75425
<i>P. amylophila</i>	YB-1287 ^T	7020		U73577
	Y-11503	5692	0	
<i>P. angophorae</i>	Y-7118 ^T	5823		U75521
	Y-11501	5830	0	
<i>P. angusta</i> (<i>Ogataea polymorpha</i>)	Y-2214 ^T	7073		U75524
<i>P. anomala</i>	Y-366 ^{NT}	5759		U74592
	Y-17813		0	
	YB-4880		0	
<i>P. antillensis</i>	Y-12881 ^T	7111		U75965
<i>P. barkeri</i>	Y-17350 ^T	7256		U75735
<i>P. (Yamadazyma) besseyi</i>	YB-4711 ^T	6343		U75729
<i>P. bimundalis</i>	Y-5343 ^T	5642		U73574
<i>P. bispora</i>	Y-1482 ^T	1890		U74589
<i>P. bovis</i>	YB-4184 ^T	2616		U75417
<i>P. (Hyphopichia) burtonii</i>	Y-1933 ^T	2352		U45712

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>P. cactophila</i>	Y-10963 ^T	6926	0	U75731
	Y-10964	6927		
	Y-12940			
<i>P. canadensis</i>	Y-1888 ^T	1992		U75415
<i>P. (Kuraisha) capsulata</i>	Y-1842 ^T	1993	0	U75516
	YB-2520			
<i>P. caribaea</i>	Y-17468 ^T	7692		U75426
<i>P. (Yamadazyma) castillae</i>	Y-7501 ^T	6053		U45769
<i>P. chambardii</i>	Y-2378 ^T	1900		U74590
<i>P. ciferrii</i>	Y-1031 ^T	111		U74587
<i>P. delftensis</i>	Y-7119 ^T	2614	0	U75721
	Y-17855	2109		
	Y-12918 ^T	7119		
<i>P. deserticola</i>	Y-10990 ^T	6154		U75422
<i>P. dryadooides</i>	Y-17232 ^T	8033	0	U73580
	Y-17233	7082		
<i>P. euphorbiae</i>	Y-17757	7083	0	
	Y-12695	7064		
<i>P. euphorbiiphila</i>	Y-12742 ^T	8083	0	U73582
	Y-1871 ^T	5640		
	Y-7553 ^T	185		
<i>P. fermentans</i>	Y-12695	7064	2	
	YB-4273 ^T	2287		
	YB-4882			
<i>P. fluxuum</i>	YB-4886		0	U75719
	Y-17349 ^T	7324		
	Y-6539			
<i>P. galeiformis</i>	Y-17348	763	0	U75738
	YB-2185 ^T	5766		
	Y-2075 ^T	2030		
<i>P. (Ogataea) glucozyma</i>	Y-324	566	0	U75520
	Y-2076	2031		
	Y-17818			
	Y-17819			
	Y-17820			
	Y-17843			
	Y-17904	2024		
	Y-17905	5256		
	Y-17970			
	YB-4128 ^T	7208		
<i>P. hampshirensis</i>	Y-17346 ^T	7521		U74598
<i>P. hangzhouana</i>	Y-7860 ^T	2028		U45770
<i>P. (Yamadazyma) haplophila</i>	Y-10967 ^T	6930		U75733
<i>P. heedii</i>	Y-7502 ^T	6139		U45713
<i>P. heimii</i>	YB-2194 ^T	5765		U75519

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>P. (Nakazawaea) holstii</i>	Y-2155 ^T	4140		U75722
<i>P. (Yamadazyma) inositovora</i>	Y-12698 ^T	8006		U45848
<i>P. jadinii</i>	Y-1542 ^T	1600		U73570
	Y-7586	621	1	
<i>P. japonica</i>	YB-2750 ^T	7209		U73579
	Y-17805	7924	2	
<i>P. kluyveri</i> var. <i>kluyveri</i>	Y-11519 ^T	188		U75727
<i>P. kluyveri</i> var. <i>cephalocereana</i>	Y-17225 ^T	7273		U75737
<i>P. kluyveri</i> var. <i>eremophila</i>	Y-17224 ^T	7272		U75736
<i>P. (Ogataea) kodamae</i>	Y-17234 ^T	7081		U75525
	Y-11843 ^{T?}		0	
<i>P. lynferdii</i>	Y-7723 ^T	6695		U74595
<i>P. (Yamadazyma) media</i>	Y-7122 ^T	5521		U45768
	Y-11524	5520	0	
<i>P. membranifaciens</i>	Y-2026 ^T	107		U75725
<i>P. methanolica</i>	Y-7685 ^T	6515		U75523
<i>P. methylivora</i>	Y-17250 ^T	7300		U75526
<i>P. (Yamadazyma) mexicana</i>	Y-11818 ^T	7066		U45797
	YB-1265		1	
<i>P. meyerae</i>	Y-17236 ^T	7076		U73578
<i>P. (Ogataea) minuta</i> var. <i>minuta</i>	Y-411 ^T	1708		U75515
<i>P. (Ogataea) minuta</i> var. <i>nonfermentans</i>	YB-2203 ^T	5764		U75518
<i>P. mississippiensis</i>	YB-1294 ^T	7023		U74597
<i>P. muscicola</i>	Y-7005 ^T	5800		U94934
<i>P. naganishii</i>	Y-7654 ^T	6429		U75724
<i>P. nakasei</i>	Y-7686 ^T	5141		U75728
<i>P. (Yamadazyma) nakazawai</i> var. <i>nakazawai</i>	Y-7903 ^T	6700		U45748
<i>P. (Yamadazyma) nakazawai</i> var. <i>akitaensis</i>	Y-7904 ^T	6701		U45766
<i>P. norvegensis</i>	Y-7687 ^T	6564		U75730
<i>P. ofunaensis</i>	Y-10998 ^T	8129		U45829
<i>P. (Yamadazyma, Kodamaea) ohmeri</i>	Y-1932 ^T	5367		U45702
	Y-2078	2037	0	
<i>P. onychis</i>	Y-7123 ^T	5587		U75421
	Y-11526	5649	2	
<i>P. opuntiae</i>	Y-11707 ^T	7010		U76203
<i>P. (Komagataella) pastoris</i>	Y-1603 ^T	704		U75963
<i>P. petersonii</i>	YB-3808 ^T	5555		U73572
<i>P. petrophilum</i>	Y-11953 ^A	7911		
	Y-11954		0	
<i>P. (Ogataea) philodendri</i>	Y-7210 ^T	6075		U75522
<i>P. (Yamadazyma) phlogaea</i>	Y-7813 ^T	6696		U45765
<i>P. pijperi</i>	YB-4309 ^T	2887		U75418
<i>P. (Ogataea) pini</i>	Y-11528 ^T	744		U75527
<i>P. populi</i>	Y-12728 ^T	8094		U75427

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>P. pseudocactophila</i>	Y-17239 ^T	6929		U75732
<i>P. quercuum</i>	YB-4281 ^T	2283		U75416
<i>P. rabaulensis</i>	Y-7945 ^T	6797		U75423
<i>P. rhodanensis</i>	Y-7854 ^T	5518		U73571
<i>P. salicaria</i>	Y-6780 ^T	5456		U75420
<i>P. (Yamadazyma) scolyti</i>	Y-5512 ^T	4802		U45788
<i>P. (Yamadazyma) segobiensis</i>	Y-11571 ^T	6857		U45742
<i>P. silvicola</i>	Y-1678 ^T	1705		U74588
<i>P. sorbitophila</i>	Y-12695 ^T	7064		
	Y-17249	8045	2	
<i>P. (Yamadazyma) spartinae</i>	Y-7322 ^T	6059		U45764
	Y-7689	6688	3	
<i>P. (Yamadazyma) stipitis</i>	Y-7124 ^T	5773		U45741
<i>P. strasburgensis</i>	Y-2383 ^T	2939		U74591
<i>P. subpelliculosa</i>	Y-1683 ^T	5767		U74593
	Y-17812		0	
<i>P. sydowiorum</i>	Y-7130 ^T	5995		U74594
<i>P. tannicola</i>	Y-17392 ^T	6065		U45803
<i>P. thermotolerans</i>	Y-11709 ^T	7012		U75962
<i>P. toletana</i>	YB-4247 ^T	2504		U75720
	YB-2508		0	
<i>P. trehalophila</i>	Y-6781 ^T	5361		U75723
<i>P. triangularis</i>	Y-5714 ^T	4094		U45796
<i>P. veronae</i>	Y-7818 ^T	6591		U73576
<i>P. wickerhamii</i>	Y-2435 ^T	4107		U75419
	Y-2436	4106	0	
<i>P. xylosa</i>	Y-12939 ^T	2286		U75718
<i>Pichia</i> sp.	Y-5377			AF017405
<i>Pichia</i> sp.	Y-5494			AF017406
<i>Pichia</i> sp.	Y-7556	2612		AF017407
<i>Pichia</i> sp.	Y-7615			AF017408
<i>Pichia</i> sp.	Y-11513	5365		AF017409
<i>Pichia</i> sp.	Y-11569	5120		AF017410
<i>Pichia</i> sp.	Y-17803	5119		AF017411
<i>Pichia</i> sp.	Y-27008			AF017412
<i>Pichia</i> sp.	YB-2076			AF017246
<i>Pichia</i> sp.	YB-4149			AF017413
<i>Protomyces</i> <i>gravidus</i>	Y-17093 ^A			U84342
<i>P. inouyei</i>	YB-4354 ^A			U84344
<i>P. inundatus</i>	Y-6349 ^A			U76528
<i>P. lactucaeabilis</i>	YB-4353 ^A			U84343
<i>P. macrosporus</i>	Y-12879 ^A			U94939
<i>P. pachydermus</i>	YB-4355 ^A			U84345
<i>Saccharomyces barnettii</i>	Y-27223 ^T	6946		U84231
<i>S. bayanus</i>	Y-12624 ^T	380		U94931
<i>S. castellii</i>	Y-12630 ^T	4309		U68557

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>S. cerevisiae</i>	Y-12631	4310	0	
	ETC NY-51		1	
	Y-12632 ^{NT}	1171		U44806
	Y-1375		0	
	Y-17732		0	
<i>S. dairenensis</i>	Y-12639 ^T	421		U68556
<i>S. exiguum</i>	Y-12640 ^{NT}	379		U68553
<i>S. kluyveri</i>	Y-12651 ^T	3082		U68552
<i>S. paradoxus</i>	Y-17217 ^{NT}	432		U68555
<i>S. pastorianus</i>	Y-1551 ^T	1538		U68547
<i>S. rosinii</i>	Y-17919 ^T	7127		U84232
<i>S. servazzii</i>	Y-12661 ^T	4311		U68558
<i>S. spencerorum</i>	Y-17920 ^T	3019		U84227
<i>S. (Pachytichospora) transvaalensis</i>	Y-17245 ^T	2186		U68549
	Y-12662	4906	2	
	YB-4106		0	
<i>S. unisporus</i>	Y-1556 ^T	398		U68554
<i>Saccharomycodes ludwigii</i>	Y-12793 ^T	821		U73601
	Y-12860	820	0	
<i>S. sinensis</i>	Y-12797 ^T	7075		U94946
<i>Saccharomycopsis capsularis</i>	Y-17639 ^{NT}	2519		U40082
	Y-7487	5638	3	
<i>S. (Endomycopella) crataegensis</i>	Y-5902 ^T	6447		U40079
<i>S. (Arthroascus) fermentans</i>	Y-17710 ^T	7830		U73600
<i>S. (Endomyces) fibuligera</i>	Y-2388 ^T	2521		U40088
	Y-11983		0	
	Y-17596	7434	0	
	YB-1043		0	
	YB-4015		0	
	YB-4806		0	
<i>S. (Arthroascus) javanensis</i>	Y-1483 ^T	2555		U40107
<i>S. malanga</i>	Y-7175 ^T	6267		U40135
	Y-11946		0	
<i>S. (Arthroascus) schoenii</i>	Y-17595 ^{NT}	7223		U40126
	YB-1542		1	
	YB-2401		1	
	YB-2404		1	
<i>S. (Guilliermondella) selenospora</i>	Y-1357 ^T	2562		U40099
	Y-17724	2563	0	
<i>S. (Botryoascus) synnaedendra</i>	Y-7466 ^T	6161		U40123
<i>S. (Endomycopella) vini</i>	Y-7290 ^T	4110		U40133
<i>Saccharomycopsis</i> sp.	Y-7404	6393		AF017725
<i>Saitoella complicata</i>	Y-17804 ^T	7301		U76530
<i>Saturnispora ahearnii</i>	Y-7555 ^T	6121		U94935
<i>S. dispora</i>	Y-1447 ^T	794		U94937
<i>S. saitoi</i>	Y-6671 ^T	4910		U94932
<i>S. zaruensis</i>	Y-7008 ^T	5799		U94933
<i>Saturnispora</i> sp.	Y-11516	5621		AF017727

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>Schizoblastosporion chiloense</i>	Y-17924 ^T	8187		U84347
<i>S. starkeyi-henricii</i>	YB-3963 ^{NT}	2159		U40089
<i>Schizosaccharomyces (Hasegawaea)</i>	Y-1361 ^T	354		U94943
<i>japonicus</i> var. <i>japonicus</i>				
<i>S. (Hasegawaea) japonicus</i> var. <i>versatilis</i>	Y-1026 ^T	103		U94938
<i>S. (Octosporomyces) octosporus</i>	Y-855 ^T	371		U76525
<i>S. pombe</i>	Y-12796 ^T	356		U40085
<i>Sporopachydermia cereana</i>	Y-7798 ^T	6644		U76529
<i>S. lactativora</i>	Y-11591 ^T	6192		U45851
<i>S. quercuum</i>	Y-17847 ^T	8070		U76532
<i>Stephanoascus ciferrii</i>	Y-10943 ^A	5295		U40138
<i>S. farinosus</i>	Y-17593 ^T	140.71		U40132
<i>S. smithiae</i>	Y-17849 ^A	7522.1		U76531
	Y-17850	7522.2	0	
<i>Sympodiomyces parvus</i>	Y-10004 ^T	6147		U40096
<i>Taphrina deformans</i>	T-857 ^A			U94948
<i>Torulaspora delbrueckii</i>	Y-866 ^{NT}	1146		U72156
<i>T. franciscae</i>	Y-17532 ^T	2926		U73604
<i>T. globosa</i>	Y-12650 ^T	764		U72166
<i>T. pretoriensis</i>	Y-17251 ^T	2187		U72157
<i>Trigonopsis variabilis</i>	Y-1579 ^T	1040		U45827
	Y-7770	4095	0	
<i>Wickerhamia fluorescens</i>	YB-4819 ^T	4565		U45719
<i>Wickerhamiella domercqiae</i>	Y-6692 ^T	4351		U45847
	YB-4574		1	
<i>Williopsis californica</i>	Y-17395 ^T	252		U75957
	Y-1680	5760	0	
	Y-6420		5	
	Y-6421	5782	5	
	Y-6432		0	
	Y-7609		1	
	Y-7610		0	
	YB-1807		0	
	YB-2757		0	
	YB-3239	5762	6	
	YB-3446		0	
	YB-3456		0	
	YB-3548		0	
	YB-3550		0	
	YB-3563		0	
	YB-4269		0	
	YB-4897		0	

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>W. mucosa</i>	YB-1344 ^T	6341		U75961
<i>W. (Komagataea) pratensis</i>	Y-12696 ^T	7079		U75964
<i>W. salicorniae</i>	Y-12834 ^T	8071		U75966
<i>W. saturnus</i> var. <i>saturnus</i>	Y-17396 ^T	254		U75958
	Y-1304	5761	0	
	Y-11764		1	
	YB-4312	2564	1	
	YB-4948		1	
<i>W. saturnus</i> var. <i>mrakii</i>	Y-1364 ^T	1707		U94929
	Y-17814		0	
<i>W. saturnus</i> var. <i>sargentensis</i>	YB-4139 ^T	6342		U94936
<i>W. saturnus</i> var. <i>suaveolens</i>	Y-17391 ^T	255		U94930
	Y-838		0	
<i>W. saturnus</i> var. <i>subsufficiens</i>	YB-1657 ^T	5763		U75960
	YB-1718		0	
	YB-3831		1	
<i>Yarrowia lipolytica</i>	YB-423 ^T	6124		U40080
	Y-1095	6317	0	
	Y-11853		0	
<i>Zygoascus hellenicus</i>	Y-17319 ^A	4099		U40125
<i>Zygosaccharomyces bailii</i>	Y-2227 ^T	680		U72161
	Y-787		0	
<i>Z. bisporus</i>	Y-12626 ^T	702		U72162
<i>Z. cidri</i>	Y-12634 ^T	4575		U84236
	Y-12635	2950	0	
<i>Z. fermentati</i>	Y-1559 ^T	707		U84239
	Y-7434	4506	0	
	Y-11844		0	
	Y-11847		0	
	Y-12620	4686	0	
	Y-17054	6544	0	
	Y-17055	6711	0	
<i>Z. florentinus</i>	Y-1560 ^T	746		U72165
	Y-12642	6081	1	
<i>Z. mellis</i>	Y-12628 ^{NT}	736		U72164
<i>Z. microellipsooides</i>	Y-1549 ^T	427		U72160
<i>Z. mrakii</i>	Y-12654 ^T	4218		U72159
	Y-12655	4219	0	
<i>Z. rouxii</i>	Y-229 ^{NT}	732		U72163
	ETC RY-208		0	
	YB-3050		0	
<i>Zygosaccharomyces</i> sp.	YB-4810			AF017728
<i>Zygozyma arxii</i>	Y-17921 ^T	7333		U84241
<i>Z. oligophaga</i>	Y-17247 ^T	7107		U45850
<i>Z. smithiae</i>	Y-17922 ^T	7407		U84242

Table 1. (Continued)

Species ^{a,b}	Strain designation ^c		No. of differences in domain D1/D2 (ca. 600 nucleotides) between type strains and conspecific isolates	GenBank accession no.
	NRRL	CBS		
<i>Z. suomiensis</i>	Y-17995	7408	0	
	Y-17356 ^T	7251		U84240
Euascomycetes				
<i>Ceratocystis fimbriata</i>	13496 ^A	146.53		U94917
<i>Emericella nidulans</i>	22233 ^A			U40122
' <i>Endomyces scopularum</i> '	Y-17633 ^A	131.86		U40092
<i>Eremascus fertilis</i>	Y-1463 ^A			U94940
<i>Neurospora crassa</i>	13141 ^A			U40124
<i>Oosporidium margaritiferum</i>	Y-1519 ^T	2531		U40090
Basidiomycete				
<i>Filobasidiella neoformans</i>	Y-170 ^A	882		U94941

^a Names in parentheses are recent proposed changes and are discussed in the text.

^b Strains listed by genus name followed by sp. are predicted to represent new species.

^c T= type strain; NT = neotype strain; A = authentic strain. NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois, USA. CBS, Centraalbureau voor Schimmelcultures, Delft/Baarn, The Netherlands.

^d *Candida magnifica*' was originally received from O. Verona and apparently was never validly described.

(5'-CTTGTTCGCTATCGGTCTC) and NL-3A (5'-GAGACCGATAGCGAACAAAG).

Sequence data were visually aligned with QEdit 2.15 (SemWare, Marietta, Ga.). Phylogenetic relationships were calculated with a Power Macintosh 8500/120 by the maximum parsimony program of PAUP 3.1.1 (Swofford, 1993) with a heuristic search employing both simple and random sequence additions. Relationships were further analyzed by the neighbor-joining program of PAUP 4.0d56 (D. L. Swofford unpublished) with the Jukes-Cantor distance measure. *Schizosaccharomyces pombe* was the designated outgroup in analyses unless otherwise indicated. Confidence limits for phylogenetic trees were estimated from bootstrap analyses (100 replications for heuristic searches and 1,000 for neighbor-joining searches). Because some regions of the nucleotide sequences were quite variable and difficult to align, analyses were made from the complete aligned dataset as well as from a second aligned dataset in which four variable sections delimited by nucleotides (reference, *Saccharomyces cerevisiae*) 122–144, 425–495, 542–563 and 603–618 were removed. The aligned datasets used for calculating phylogenetic trees are available from us as computer files.

Nucleotide sequence accession numbers

The nucleotide sequences determined in this study have been deposited with GenBank under the accession numbers shown in Table 1.

Nuclear DNA reassessments

nDNA complementarity between strains was measured spectrophotometrically as described by Kurtzman et al. (1980a).

Results and discussion

Resolution of species

Peterson & Kurtzman (1991) determined the extent of rRNA nucleotide divergence in LSU domain D2 for closely related species pairs of *Issatchenka*, *Pichia*, and *Saccharomyces* that earlier had been characterized either from genetic crosses or by nDNA reassociation. Their work showed that nucleotide substitutions in the D2 domain do not generally exceed 1% among conspecific strains, and they predicted that isolates showing a greater divergence would be members of different species. We expanded these comparisons to include additional heterothallic species as well as

Table 2. Extent of DNA relatedness and LSU domain D1/D2 rDNA nucleotide divergence between type strains of closely related yeasts

NRRL No.	Taxa ^a	% nDNA	rDNA
		Reassociation (Reference) ^b	Nucleotide Differences ^c
Y-17643	<i>Candida bertae</i> var. <i>bertae</i>		
Y-17646	<i>Candida bertae</i> var. <i>chiloensis</i>	98 (1)	1
Y-17084	<i>Candida etchellsii</i>		
Y-6694	<i>Candida halonitratophila</i>	84 (2)	1
Y-2484	<i>Candida nodaensis</i>	89 (2)	0
Y-2483	<i>Candida halophila</i>		
Y-7226	<i>Candida mannitofaciens</i>	99 (2)	1
Y-12858	<i>Candida shehatae</i> var. <i>shehatae</i>		
Y-12854	<i>Candida shehatae</i> var. <i>insectosa</i>	49 (3)	1
Y-12856	<i>Candida shehatae</i> var. <i>lignosa</i>	46 (3)	0
Y-12854	<i>Candida shehatae</i> var. <i>insectosa</i>		
Y-12856	<i>Candida shehatae</i> var. <i>lignosa</i>	59 (3)	1
Y-6652	<i>Candida versatilis</i>		
Y-2483	<i>Candida halophila</i>	83 (2)	1
Y-7226	<i>Candida mannitofaciens</i>	91 (2)	2
Y-6665	<i>Debaryomyces tamarii</i>	90 (2)	1
Y-7426	<i>Debaryomyces hansenii</i> var. <i>hansenii</i>		
Y-17914	<i>Debaryomyces hansenii</i> var. <i>fabryi</i>	52 (4)	2
Y-7425	<i>Debaryomyces couderpii</i>	16 (5)	4
Y-2171	<i>Debaryomyces maramus</i>	8 (5)	7
Y-7108	<i>Debaryomyces nepalensis</i>	15 (5)	6
Y-6670	<i>Debaryomyces robertsiae</i>	6 (6)	6
Y-7585	<i>Debaryomyces melissophilus</i>		
Y-7804	<i>Debaryomyces</i> sp.	32 (7)	2
Y-10	<i>Debaryomyces occidentalis</i> var. <i>occidentalis</i>		
Y-7400	<i>Debaryomyces occidentalis</i> var. <i>persoonii</i>	83 (5)	2
Y-2022	<i>Debaryomyces polymorphus</i>		
Y-7423	<i>Debaryomyces castellii</i>	20 (5)	5
YB-4229	<i>Debaryomyces pseudopolymorphus</i>	21 (5)	3
Y-7430	<i>Debaryomyces vanrijiae</i> var. <i>vanrijiae</i>	24 (5)	8
Y-7535	<i>Debaryomyces vanrijiae</i> var. <i>yarrowii</i>		7
Y-7430	<i>Debaryomyces vanrijiae</i> var. <i>vanrijiae</i>		
Y-7535	<i>Debaryomyces vanrijiae</i> var. <i>yarrowii</i>	68 (5)	1
Y-1613	<i>Hanseniaspora osmophilica</i>		
Y-17529	<i>Hanseniaspora vineae</i>	47 (8)	7
Y-7663	<i>Issatchenka scutulata</i> var. <i>scutulata</i>		
Y-10920	<i>Issatchenka scutulata</i> var. <i>exigua</i>	21 (9)	27
Y-8279	<i>Kluyveromyces lactis</i> var. <i>lactis</i>		
Y-8278	<i>Kluyveromyces lactis</i> var. <i>drosophilicola</i>	80 (10)	0
Y-8281	<i>Kluyveromyces marxianus</i>	10 (10)	1
Y-11553	<i>Lipomyces kononenkoae</i> subsp. <i>kononenkoae</i>		
Y-7042	<i>Lipomyces kononenkoae</i> subsp. <i>spencer-martinsiae</i>	51 (11)	10
Y-11557	<i>Lipomyces starkeyi</i>	11 (11)	6
Y-12810	<i>Nadsonia fulvescens</i> var. <i>fulvescens</i>		
Y-1568	<i>Nadsonia fulvescens</i> var. <i>elongata</i>	70 (12)	1

Table 2. (Continued)

NRRL No.	Taxa ^a	% nDNA Reassociation (Reference) ^b	rDNA Nucleotide Differences ^c
Y-2156	<i>Pichia americana</i>		
Y-5343	<i>Pichia bimundalis</i>	19 (13)	2
Y-10978	<i>Pichia amethionina</i> var. <i>amethionina</i>		
Y-10981	<i>Pichia amethionina</i> var. <i>pachycereana</i>	70 (14)	15
Y-17468	<i>Pichia caribaea</i>	40 (14)	23
YB-1287	<i>Pichia amylophila</i>		
YB-1294	<i>Pichia mississippiensis</i>	27 (15)	4
Y-12881	<i>Pichia antillensis</i>		
Y-11707	<i>Pichia opuntiae</i>	55 (16)	33
Y-10963	<i>Pichia cactophila</i>		
Y-17239	<i>Pichia pseudocactophila</i>	33 (17)	20
Y-7553	<i>Pichia farinosa</i>		
Y-12695	<i>Pichia sorbitophila</i>	74 (18)	0
Y-17649	<i>Candida cacaoi</i>	87 (18)	0
Y-1542	<i>Pichia jadinii</i>		
Y-7586	<i>Candida utilis</i>	85 (19)	1
Y-11519	<i>Pichia kluyveri</i> var. <i>kluyveri</i>		
Y-17225	<i>Pichia kluyveri</i> var. <i>cephalocereana</i>	72 (20)	3
Y-17224	<i>Pichia kluyveri</i> var. <i>eremophila</i>	66 (20)	8
Y-17350	<i>Pichia barkeri</i>	20 (21)	20
Y-411	<i>Pichia minuta</i> var. <i>minuta</i>		
YB-2203	<i>Pichia minuta</i> var. <i>nonfermentans</i>	49 (22)	7
Y-7903	<i>Pichia nakazawae</i> var. <i>nakazawae</i>		
Y-7904	<i>Pichia nakazawae</i> var. <i>akitaensis</i>	41 (13)	2
Y-11571	<i>Pichia segobiensis</i>		
Y-7124	<i>Pichia stipitis</i>	38 (23)	2
Y-17392	<i>Pichia tannicola</i>		
Y-7499	<i>Pichia abadieae</i>	100 (23)	0
YB-4247	<i>Pichia toletana</i>		
Y-12939	<i>Pichia xylosa</i>	29 (24)	1
Y-12624	<i>Saccharomyces bayanus</i>		
Y-12693	<i>Saccharomyces pastorianus</i>	72 (25)	0
Y-17217	<i>Saccharomyces paradoxus</i>	22 (26)	8
Y-12632	<i>Saccharomyces cerevisiae</i>		
Y-12624	<i>Saccharomyces bayanus</i>	10 (25)	12
Y-12693	<i>Saccharomyces pastorianus</i>	57 (25)	12
Y-17217	<i>Saccharomyces paradoxus</i>	50 (26)	6
Y-6671	<i>Saturnispora saitoi</i>		
Y-7555	<i>Saturnispora ahearnii</i>	30 (27)	12
Y-17849	<i>Stephanoascus smithiae</i>		
Y-17083	<i>Candida edax</i>	95 (28)	0
Y-866	<i>Torulaspora delbrueckii</i>		
Y-17251	<i>Torulaspora pretoriensis</i>	13 (5)	5
Y-17396	<i>Williopsis saturnus</i> var. <i>saturnus</i>		
Y-1364	<i>Williopsis saturnus</i> var. <i>mrakii</i>	52 (27)	1

Table 2. (Continued)

NRRL No.	Taxa ^a	% nDNA Reassociation (Reference) ^b	rDNA Nucleotide Differences ^c
YB-4139	<i>Williopsis saturnus</i> var. <i>sargentensis</i>	43 (27)	0
Y-17391	<i>Williopsis saturnus</i> var. <i>suaveolens</i>	72 (27)	0
YB-1657	<i>Williopsis saturnus</i> var. <i>subsufficiens</i>	56 (27)	5

^a Strains showing 70–100% nDNA relatedness are regarded as conspecific; strain pairs with 40–70% nDNA relatedness are considered either as varieties or as sister species depending on any demonstrated fertility between the pairs.

^b nDNA reassociation data are from: (1) Tengku Zainal Mulok, 1988; (2) Suzuki et al., 1992; (3) Kurtzman, 1990; (4) Nakase & Suzuki, 1985; (5) Price et al., 1978; (6) Kurtzman & Robnett, 1994b; (7) Kurtzman & Robnett, 1991; (8) Meyer et al., 1978; (9) Kurtzman et al., 1980b; (10) Fuson et al., 1987; (11) Smith et al., 1995a; (12) Golubev et al., 1989; (13) Kurtzman, 1984b; (14) Phaff et al., 1992; (15) Kurtzman et al., 1980a; (16) Starmer et al., 1984; (17) Holzschu et al., 1983; (18) Lee et al., 1992; (19) Kurtzman et al., 1979; (20) Phaff et al., 1987b; (21) Phaff et al., 1987a; (22) Kurtzman, 1984a; (23) present study; (24) Kurtzman, 1992; (25) Vaughan-Martini & Kurtzman, 1985; (26) Vaughan-Martini, 1989; (27) Kurtzman, 1991; (28) Giménez-Jurado et al., 1994.

^c Based on ca. 600 nucleotides in domain D1/D2.

some homothallic and anamorphic taxa. Of the ca. 500 species and varieties listed in Table 1, 103 are represented by two or more strains. Strain variation among most of those species ranged from 0–2 nucleotides. However, strains of three of the species showed up to 3 nucleotide differences, and the mating types of *Metschnikowia agaves* differed by 5 nucleotides. The 17 strains of *Williopsis californica* examined ranged from 0–6 differences, but the 5–6 differences shown by three strains are contiguous deletions rather than substitutions.

To further test for recognition of species from extent of nucleotide substitutions, taxa with reduced nDNA relatedness were examined (Table 2). Strain pairs with less than 30% nDNA relatedness generally have greater than 3 nucleotide differences and would be recognized as separate species in accord with the comparisons given in Table 1. *Saccharomyces bayanus* and *S. pastorianus* have no sequence differences and are an exception. It has been proposed that *S. pastorianus* is a partial amphidiploid that arose from hybridization between *S. cerevisiae* and *S. bayanus* and has retained the rRNA genes of the latter species (Peterson & Kurtzman 1991). *Kluyveromyces lactis*/*K. marxianus*, *Pichia segobiensis*/*P. stipitis* and *P. toletana*/*P. xylosa* represent sister species that differ from each other by just 1–2 nucleotides. Other taxon pairs, such as *Issatchenkovia scutulata* var. *scutulata*/var. *exigua*, *Pichia antillensis*/*P. opuntiae* and *P. cactophila*/*P. pseudocactophila* show greater rDNA divergence than would be expected from their relatively high

nDNA relatedness. Most of these latter pairs are members of the same clade, which suggests that the rate for D1/D2 substitutions in this group should be examined in greater detail.

Taking into account the variation seen in the preceding comparisons, it is predicted that strains showing greater than 1% substitutions in the ca. 600-nucleotide D1/D2 domain are likely to be different species and that strains with 0–3 nucleotide differences are either conspecific or sister species. From this correlation, 55 currently accepted yeast species and varieties appear either conspecific or as sister species of earlier described species (Table 3). These predictions will be tested in the future by nDNA reassociation. The proposed conspecific taxa are similar in their reactions on standard physiological tests except for *Candida sake*/*C. austromarina* (Kurtzman & Robnett, 1997). Barnett et al. (1990) reported *C. sake* to be more fermentative than *C. austromarina*, to assimilate a larger number of carbon compounds and to have an optimum growth temperature at least 5 °C greater. Consequently, the predicted conspecificity of this species pair needs to be verified by nDNA reassociation.

Relationships among species and genera

Phylogenetic analyses of 5S, 18S and 26S domain D1/D2 rRNA/rDNA nucleotide sequences have each demonstrated that members of the ascomycetes separate into three major lineages: (1) the Hemias-

Table 3. Predicted relatedness among described yeast species with similar or identical nucleotide sequences in LSU rDNA domain D1/D2.

NRRL No.	Species pairs ^{a,b}	rDNA nucleotide differences ^c	Predicted relatedness
Y-6732	<i>Ambrosiozyma platypodis</i>		
Y-7524	<i>Ambrosiozyma ambrosiae</i>	2	Same/sister species
Y-17632	<i>Ascoidea africana</i>		
Y-17576	<i>Ascoidea corymbosa</i>	0	Same species
YB-3897	<i>Candida aaseri</i>		
Y-17648	<i>Candida butyri</i>	0	Same species
Y-17641	<i>Candida anatomiae</i>		
Y-17681	<i>Candida populi</i>	2	Same/sister species
Y-2332	<i>Candida boidinii</i>		
Y-17661	<i>Candida ooitensis</i>	0	Same species
Y-17080	<i>Candida boleticola</i>		
Y-17656	<i>Candida laureliae</i>	1	Same species
Y-17666	<i>Candida ralunensis</i>	1	Same species
Y-17071	<i>Candida chiropterorum</i>		
Y-17704	<i>Arxula terrestris</i>	4	Sister species
Y-7589	<i>Candida diddensiae</i>		
Y-10942	<i>Candida naeodendra</i>	0	Same species
Y-17072	<i>Candida fructus</i>		
Y-17088	<i>Candida musae</i>	0	Same species
Y-6949	<i>Candida glaebosa</i>		
Y-17316	<i>Candida saitoana</i>	4	Sister species
Y-17911	<i>Candida pseudoglaebosa</i>	3	Same/sister species
Y-17074	<i>Candida humilis</i>		
Y-7245	<i>Candida milleri</i>	1	Same species
Y-981	<i>Candida intermedia</i>		
Y-10939	<i>Candida pseudointermedia</i>	3	Same/sister species
Y-12697	<i>Candida paludigena</i>		
Y-17329	<i>Candida castrensis</i>	1	Same species
Y-17663	<i>Candida petrohuensis</i>		
Y-17327	<i>Candida ancudensis</i>	0	Same species
Y-17675	<i>Candida drimydis</i>	0	Same species
Y-1622	<i>Candida sake</i>		
Y-17769	<i>Candida austromarina</i>	0	Same species
Y-6656	<i>Candida santamariae</i> var. <i>santamariae</i>		
Y-17758	<i>Candida beechii</i>	0	Same species
Y-17647	<i>Candida santamariae</i> var. <i>membranifaciens</i>	2	Same/sister species
Y-11998	<i>Candida succiphila</i>		
Y-17856	<i>Candida cellulolytica</i>	0	Same species
Y-17658	<i>Candida methanolophaga</i>	0	Same species
Y-1498	<i>Candida tenuis</i>		
Y-17708	<i>Mastigomyces philippovii</i>	1	Same species
Y-17670	<i>Candida tepae</i>		
Y-17673	<i>Candida antillancae</i>	0	Same species
Y-17328	<i>Candida bondarzewiae</i>	0	Same species
Y-6660	<i>Candida viswanathii</i>		
Y-17317	<i>Candida lodderae</i>	2	Same/sister species

Table 3. (Continued)

NRRL No.	Species pairs ^{a,b}	rDNA nucleotide differences ^c	Predicted relatedness
Y-1774	<i>Candida zeylanoides</i>		
Y-17086	<i>Candida krissii</i>	0	Same species
Y-17580	<i>Dipodascus armillariae</i>		
Y-17609	' <i>Endomyces decipiens</i> '	1	Same species
Y-17574	<i>Dipodascus ovetensis</i>		
Y-17575	<i>Dipodascus ambrosiae</i>	0	Same species
Y-17578	<i>Dipodascus spicifer</i>		
Y-17570	<i>Geotrichum clavatum</i>	1	Same species
Y-7112	<i>Metschnikowia reukaufii</i>		
Y-5717	' <i>Candida magnifica</i> '	2	Same/sister species
Y-1568	<i>Nadsonia fulvescens</i> var. <i>elongata</i>		
Y-12797	<i>Saccharomyces sinensis</i>	0	Same species
Y-10963	<i>Pichia cactophila</i>		
Y-2029	<i>Candida inconspicua</i>	2	Same/sister species
Y-12918	<i>Pichia deserticola</i>		
Y-12615	<i>Candida ethanolica</i>	2	Same/sister species
Y-7553	<i>Pichia farinosa</i>		
Y-7478	<i>Debaryomyces halotolerans</i> ^d	2	Same species
Y-11953	<i>Pichia petrophilum</i> ^d	0	Same species
YB-4273	<i>Pichia fluxuum</i>		
Y-1615	<i>Candida vini</i>	0	Same species
Y-2075	<i>Pichia (Candida) guilliermondii</i>		
Y-17857	<i>Candida fukuyamaensis</i>	1	Same species
Y-17685	<i>Candida xestobii</i>	1	Same species
Y-7502	<i>Pichia heimii</i>		
Y-2594	<i>Candida rhagii</i>	2	Same/sister species
Y-2155	<i>Pichia holstii</i>		
Y-2028	<i>Candida ernobii</i>	2	Same/sister species
Y-17655	<i>Candida karawaiewii</i>	2	Same/sister species
Y-17250	<i>Pichia methylivora</i>		
Y-11996	<i>Candida cariosilignicola</i>	2	Same/sister species
Y-11818	<i>Pichia mexicana</i>		
Y-17672	<i>Candida veronae</i> ^e	0	Same species
Y-11528	<i>Pichia pini</i>		
YB-2194	<i>Pichia henricii</i>	3	Same/sister species
Y-7945	<i>Pichia rabaulensis</i>		
Y-17760	<i>Candida odintsovae</i>	3	Same/sister species
Y-1678	<i>Pichia silvicola</i>		
Y-7005	<i>Pichia muscicola</i>	2	Same/sister species
Y-1683	<i>Pichia subpelluculosa</i>		
Y-17244	<i>Hansenula arabitolgenes</i>	0	Same species
Y-7723	<i>Pichia lynferdii</i>	3	Same/sister species
Y-12879	<i>Protomyces macrosporus</i>		
Y-6349	<i>Protomyces inundatus</i>	1	Same species
YB-4354	<i>Protomyces inouyei</i>		
YB-4353	<i>Protomyces lactucaedebilis</i>	2	Same/sister species
YB-4355	<i>Protomyces pachydermus</i>	3	Same/sister species

Table 3. (Continued)

NRRL No.	Species pairs ^{a,b}	rDNA nucleotide differences ^c	Predicted relatedness
Y-7555	<i>Saturnispora ahearnii</i>		
YB-4711	<i>Pichia besseyi</i>	2	Same/sister species
Y-7008	<i>Saturnispora zaruensis</i>		
Y-17640	<i>Candida agrestis</i> ^f	0	Same species
Y-6591	<i>Zygoascus hellenicus</i>		
Y-17346	<i>Pichia hangzhouana</i>	0	Same species

^a Comparisons were made with the type strains of the species listed.

^b The species first listed in each pair or group has taxonomic priority if the taxa are conspecific.

^c Based on ca. 600 nucleotides in domain D1/D2.

^d The extent of nDNA relatedness between the type strain of *Pichia farinosa* and the type strains of *Debaryomyces halotolerans* and *P. petrophilum* is 100% and 72%, respectively (present study).

^e Lee et al. (1993) demonstrated from nDNA relatedness that *C. veronae*, *C. entomaea* and *C. terebra* are conspecific. All have identical D1/D2 sequences.

^f T. Nakase (personal communication) has suggested that CBS 8055 (NRRL Y-17640) does not represent *Candida agrestis*.

comycetes (Order Saccharomycetales), which include budding yeasts and yeastlike taxa such as *Ascoidea* and *Cephaloascus*; ascii of this group are not formed in or on fruiting bodies, (2) the Euascomycetes, a sister group to the Hemiascomycetes, represent the 'filamentous' species, some of which are dimorphic; ascii of nearly all species form within or upon fruiting bodies, and (3) the 'Archiascomycetes', a phylogenetically broad assemblage of yeastlike taxa basal to the preceding two groups and comprised of the genera *Schizosaccharomyces*, *Saitoella*, *Protomyces*, *Taphrina* and *Pneumocystis* (Barns et al., 1991; Bruns et al., 1991; Eriksson et al., 1993; Hausner et al., 1992; Hendriks et al., 1992; Kurtzman 1993a,b; Kurtzman & Robnett, 1991, 1994a, 1995, 1997; Liu & Kurtzman, 1991; Nishida & Sugiyama, 1993; Walker, 1985; Wilmette et al., 1993).

The species included in the present study were initially separated into clades by analyzing the entire dataset by neighbor-joining and by a simple heuristic search from maximum parsimony. Both trees were similar and placed all currently accepted species in the Saccharomycetales. The deletion of highly variable areas from analysis, as described earlier, had essentially no effect on branching order. We further tested whether a species phylogeny based on D1/D2 sequences was concordant with an 18S rDNA gene tree because the 18S gene sequence is widely used in phylogenetic studies. James et al. (1997) determined

complete 18S rDNA sequences for many members of the *Saccharomyces* clade, providing us with the opportunity to make a comparison of phylogenies derived from the two genes. The analysis (Figure 1) shows that D1/D2 sequences provide somewhat greater resolution of terminal lineages than does the 18S gene but that species relationships are quite similar in both trees when branches have strong bootstrap support. The major exception is *Zygosaccharomyces mrankii*, which is near *Saccharomyces florentinus* in the D1/D2 tree but is a member of the *Torulaspora* clade in the 18S tree. Confidence in phylogenetic analysis was assessed by Hillis & Bull (1993) who stated that under conditions of equal rates of change, symmetric phylogenies, and internodal change of $\leq 20\%$ of the characters, bootstrap proportions of $\geq 70\%$ usually correspond to a probability of $\geq 95\%$ that the corresponding clade is real. In contrast, poorly supported lineages represent only a weak hypothesis of species relationships. Because support for basal lineages is weak in gene trees derived from domain D1/D2, as well as from 18S sequences, we make no proposals for redefining genera, but we suggest that many present genera are not monophyletic and that additional genes must be compared before yeast classification can have a solid phylogenetic basis.

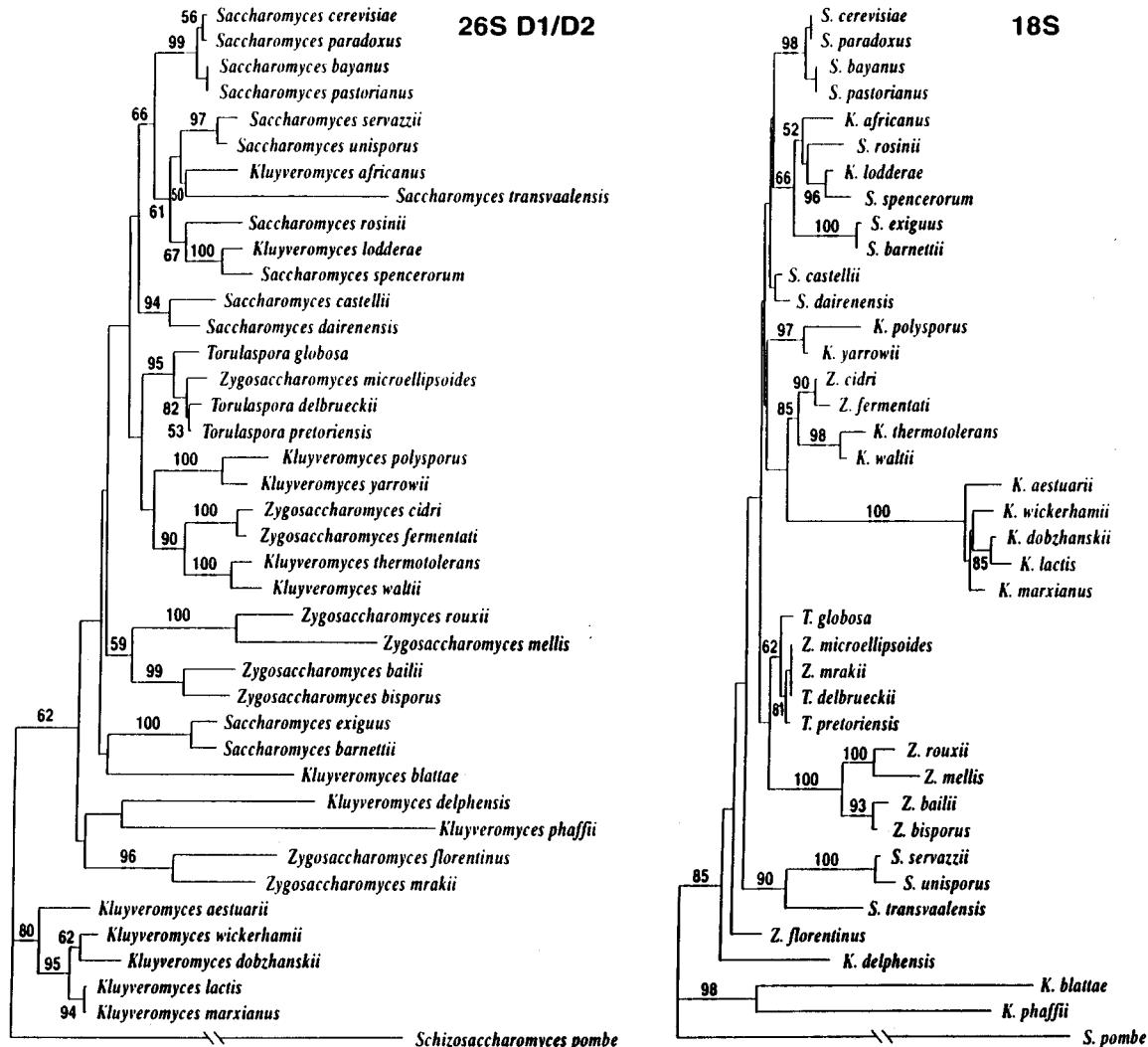


Figure 1. Phylogenetic trees calculated from neighbor-joining depicting relationships among type strains of selected species of the *Saccharomyces* clade analyzed from LSU 26S domain D1/D2 rDNA and from 18S rDNA. Branch lengths are proportional to nucleotide differences, and the numbers given at nodes are the percentage of frequencies with which a given branch appeared in 1000 bootstrap replications. Frequencies under 50% are not given. 26S D1/D2: 1 of 4 most parsimonious trees, tree length = 711, consistency index (CI) = 0.525, retention index (RI) = 0.611, rescaled consistency index (RC) = 0.321, homoplasy index (HI) = 0.475, number of parsimony-informative characters = 147. 18S: 1 of 100 most parsimonious trees, tree length = 491, CI = 0.648, RI = 0.694, RC = 0.450, HI = 0.352, number of parsimony-informative characters = 125. The branch for outgroup species *Schizosaccharomyces pombe* is half actual length in both trees. Note that species positions are not always concordant between trees when branches are weakly supported. For a further comparison of this effect, see Figure 2.

Saccharomyces clade

From the phylogenetic analysis shown in Figure 2, the *Saccharomyces* clade includes known species of *Saccharomyces*, *Arxiozyma*, *Eremothecium*, *Hanseniaspora* (anamorph, *Kloeckera*), *Kluyveromyces*, *Torulaspora*, *Zygosaccharomyces*, *Saccharomyces* and several species of *Candida*, including *C. humilis* and its proposed synonym *C. milleri*, which are common to naturally fermented foods and beverages. Species of

the genera *Eremothecium* and *Hanseniaspora* (*Kloeckera*-*eraspora*, synonym *pro parte*, Yamada et al., 1992c) were expected to form distinct subclades as seen from earlier studies of the D1/D2 domain (Boekhout et al., 1994; Kurtzman, 1995). Messner et al. (1995) proposed that *Eremothecium* is a member of the *Saccharomycetaceae* whereas Kurtzman (1995) placed it in a separate family, the *Eremotheciaceae*. The issue of family assignment is unresolved by the present

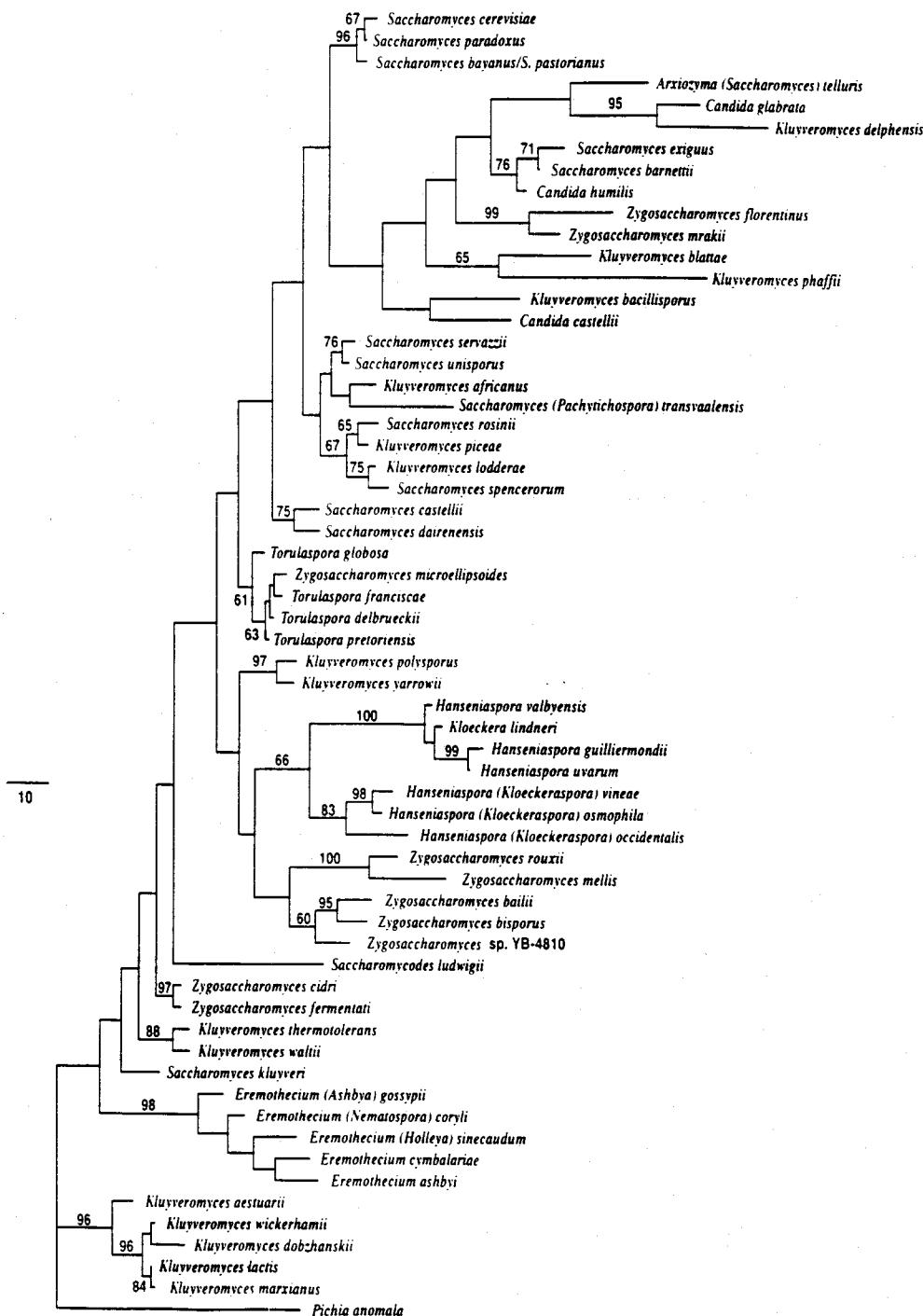


Figure 2. Phylogenetic tree of the *Saccharomyces* clade represented by 1 of 60 most parsimonious trees derived from maximum parsimony analysis of LSU domain D1/D2. Branch lengths are proportional to nucleotide differences as indicated on the marker bar. Numbers given at nodes are the percentage of frequencies with which a given branch appeared in 100 bootstrap replicates. Frequencies under 50% are not given. Tree length = 1044, CI = 0.375, RI = 0.613, RC = 0.230, HI = 0.625. Each species is represented by the type strain. Genus names given in parentheses represent alternative classifications. The outgroup species in this analysis was *Pichia anomala*, which gave a tree 64 steps shorter than that produced with *Schizosaccharomyces pombe* as outgroup.

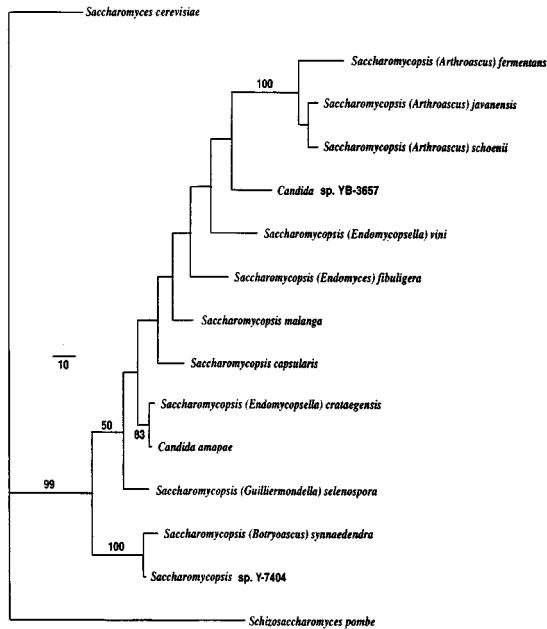


Figure 3. Phylogenetic tree of the genus *Saccharomyopsis* represented by the single most parsimonious tree derived from maximum parsimony analysis. Tree length = 433, CI = 0.711, RI = 0.598, RC = 0.425, HI = 0.289. *Saccharomyopsis capsularis* is the type species of the genus.

dataset. When analyzed with species in Figure 2, *Eremothecium* groups within the Saccharomycetaceae, but when all ca. 500 ascomycetous species are included in the analysis, *Eremothecium* becomes basal to the *Saccharomyces* clade. As discussed earlier, James et al. (1997) were unable to resolve the genera *Saccharomyces*, *Kluyveromyces*, *Torulaspora* and *Zygosaccharomyces* from analysis of 18S rDNA sequences. We combined the 18S rDNA sequences of James et al. (1997) with our D1/D2 sequences and obtained bootstrap values as much as 20% higher for some of the weaker nodes (data not shown), but the resolution was still insufficient to resolve confidently the basal lineages required for circumscription of genera.

Saccharomyopsis

Species of *Saccharomyopsis* are characterized by multilateral budding and septate hyphae. Ascospores differ considerably among species and may be hat-shaped (galeate), spheroidal to elongate, with or without equatorial ledges, or short polar appendages may be formed. This variation in ascospore shape has led to a proliferation of genera. Kurtzman & Robnett (1995) proposed from analysis of D1/D2 sequences that *Arthroascus*, *Endomycopsella*, *Guilliermondella* and *Botryoascus* represent synonyms of *Saccharomyces*,

a conclusion supported by the present expanded dataset (Figure 3). *Candida amapae* was included in the current study and is seen to be closely related to *S. crataegensis*.

Ascoidea, Dipodascus, Galactomyces, Nadsonia

Kurtzman & Robnett (1995) demonstrated from analysis of D1/D2 sequences that species of *Dipodascus* and its anamorph *Geotrichum* separate into two closely related clades, one of which includes species of *Galactomyces*. In the present study, we show that the species described as *Schizoblastosporion chiloense* is near *Dipodascus ingens* and should be transferred to the anamorphic genus *Geotrichum* (Figure 4). In addition, the data indicate that the following taxon pairs may be conspecific: *Dipodascus armillariae*/‘*Endomyces decipiens*’; *D. ovatus*/*D. ambrosiae* and *D. spicifer*/*Geotrichum clavatum* (Table 3). The analysis provides no support for maintenance of *Galactomyces* as a separate genus.

Ascoidea and *Nadsonia* appear related to *Dipodascus* (Figure 4). The anamorphic *Schizoblastosporion starkeyi-henricii* is a member of the *Nadsonia* clade, and *Saccharomyces sinensis* was found to be conspecific with *Nadsonia fulvescens* var. *elongata* (no nucleotide differences, Table 3). In this analysis, As-

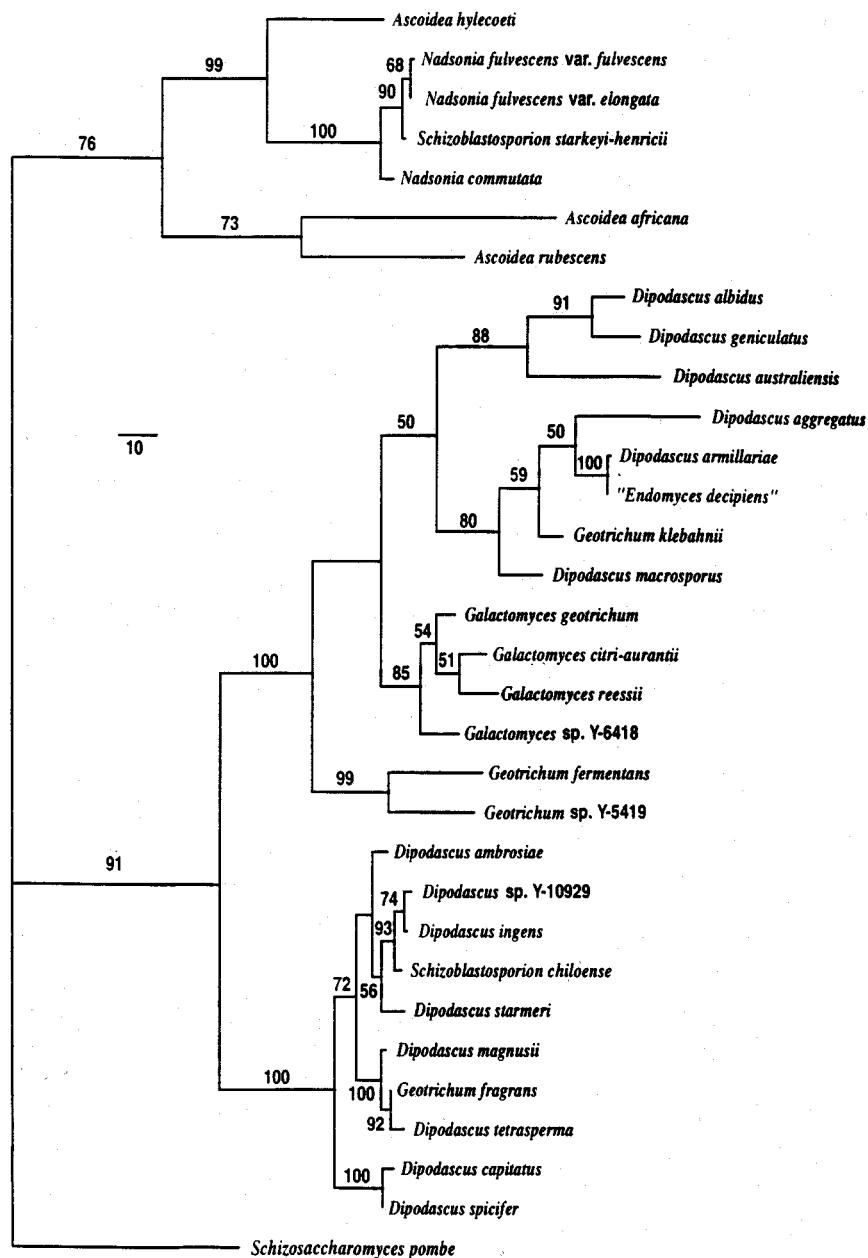


Figure 4. Phylogenetic tree of the Ascoidea/Nadsonia/Dipodascus clade represented by 1 of 2 most parsimonious trees derived from maximum parsimony analysis. Tree length = 968, CI = 0.555, RI = 0.751, RC = 0.416, HI = 0.445. *Schizoblastosporion starkeyi-henricii* is a member of the Nadsonia clade whereas *S. chiloense* is closely related to *Dipodascus ingens*. Phaff et al. (1997) found 98% nDNA relatedness between *Dipodascus ingens* and *Pichia humboldtii* (*Dipodascus* sp. NRRL Y-10929), thus demonstrating the two taxa to be conspecific. The strains differ by 3 nucleotides.

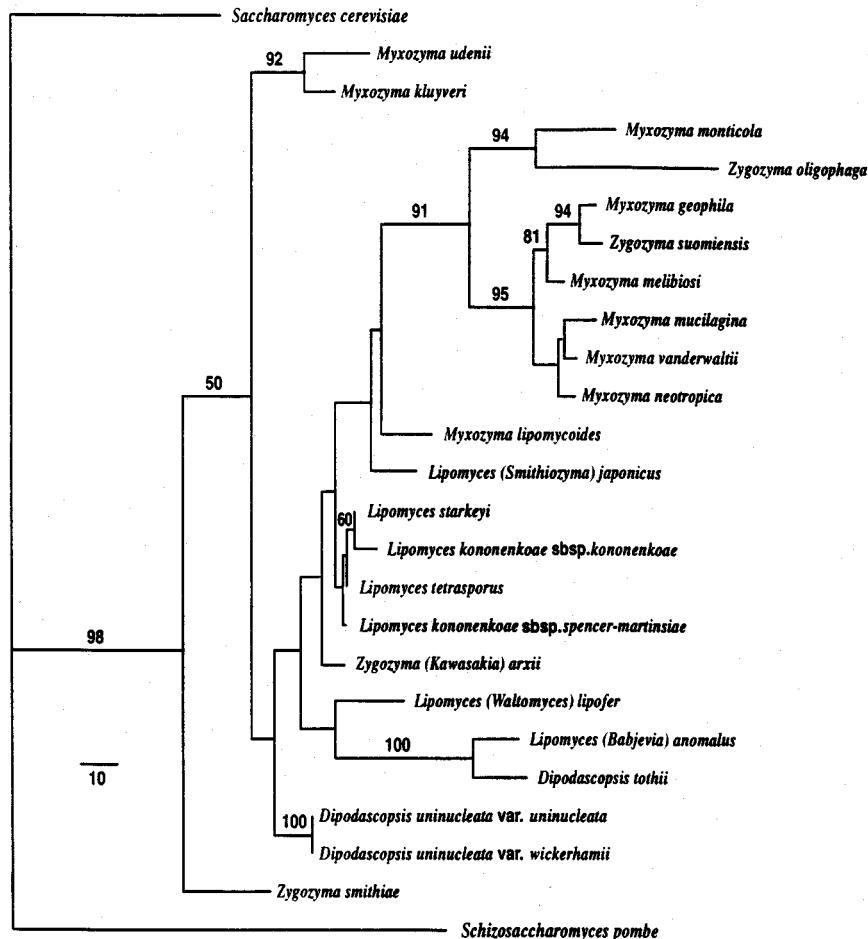


Figure 5. Phylogenetic tree of the *Lipomyces* clade represented by 1 of 7 most parsimonious trees derived from maximum parsimony analysis. Tree length = 532, CI = 0.607, RI = 0.617, RC = 0.375, HI = 0.393.

coidea hylecoeti is rather divergent from *A. africana* and *A. rubescens*.

The genera *Nadsonia*, *Wickerhamia*, *Hanseniaspora* and *Saccharomyces* share the unique morphological property of bipolar budding, i.e., buds form only at the ends of cells. However, this character appears not to predict a common evolutionary origin. *Hanseniaspora* and *Saccharomyces* are members of the *Saccharomyces* clade (Figure 2), *Nadsonia* is associated with *Ascoidea* (Figure 4) and *Wickerhamia* is basal to *Debaryomyces* (Figure 9).

Lipomyces clade

Lipomyces (proposed anamorph *Myxozyma*), *Zygozyma* and *Dipodascopsis* are placed in the family Lipomycetaceae (Cottrell & Kock, 1989; van der Walt et al., 1987). Unifying characters include similar cel-

lular fatty acids and the production of extracellular starch-like compounds. *Zygozyma* is noted for ascospores that arise following conjugation either between cellular protuberances or between individual cells, a characteristic less common for *Lipomyces*. *Dipodascopsis* has acicular or cylindrical ascospores that may form 30 to 100 or more ascospores. Members of the Lipomycetaceae have coenzyme Q (ubiquinone) with 8 to 10 isoprene units in the side chain, and many of the species tend to have uniquely ornamented ascospores. Various combinations of ascospore ornamentation and number of isoprene units in coenzyme Q have been used to realign members of the family resulting in the description of *Waltomyces* (Yamada & Nakase, 1985), *Smithiozyma* (Kock et al., 1995), *Babjevia* (Smith et al., 1995b) and *Kawasakiia* (Yamada & Nogawa, 1995).

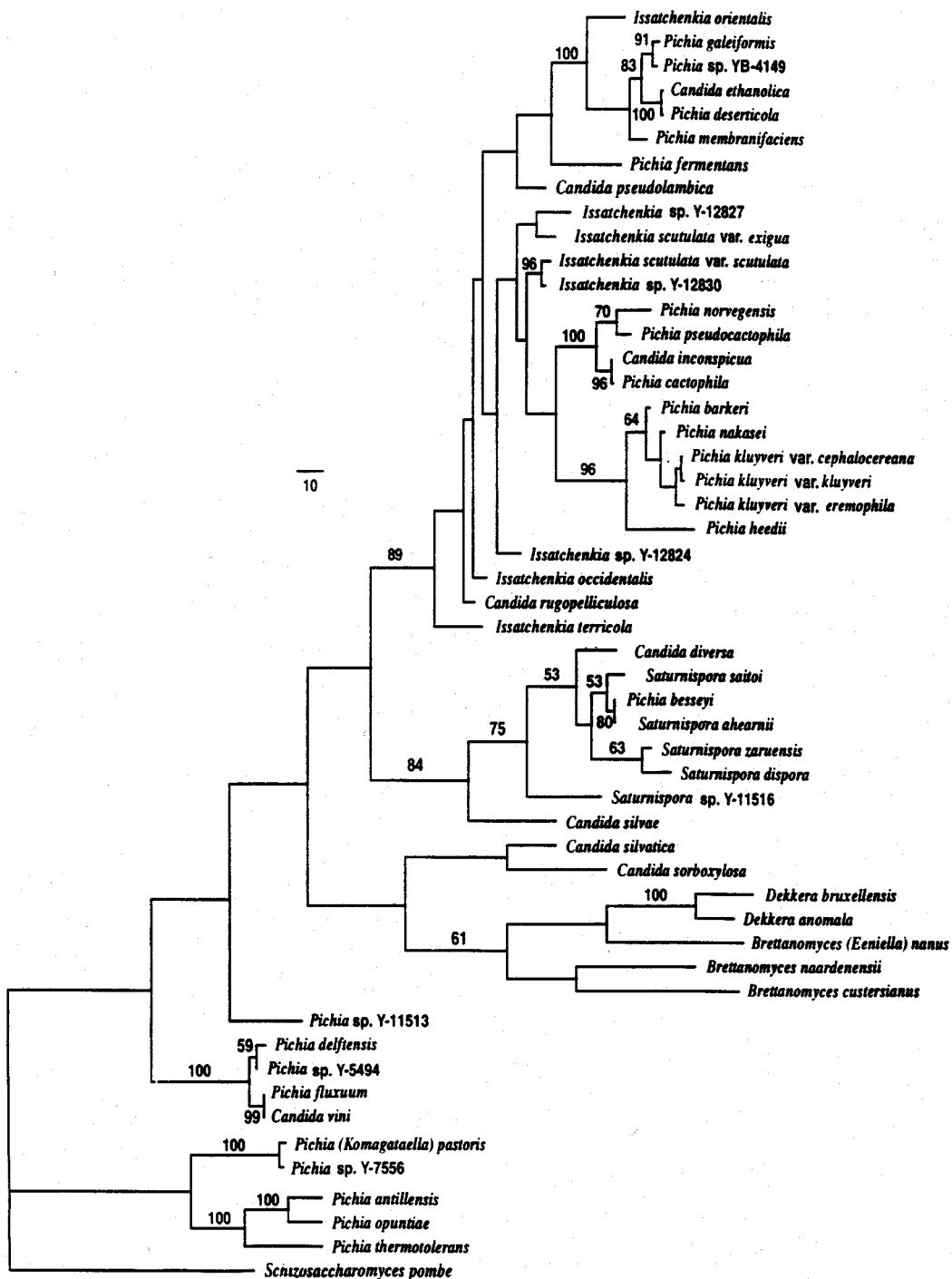


Figure 6. Phylogenetic tree of the *Pichia*/*Issatchenkia*/*Saturnispora*/*Dekkera* clade represented by 1 of 2 most parsimonious trees derived from maximum parsimony analysis. Tree length = 1677, CI = 0.439, RI = 0.691, RC = 0.303, HI = 0.561. *Pichia pastoris* and *Pichia* sp. NRRL Y-7556 are the only species included in the phylogram that assimilate methanol.

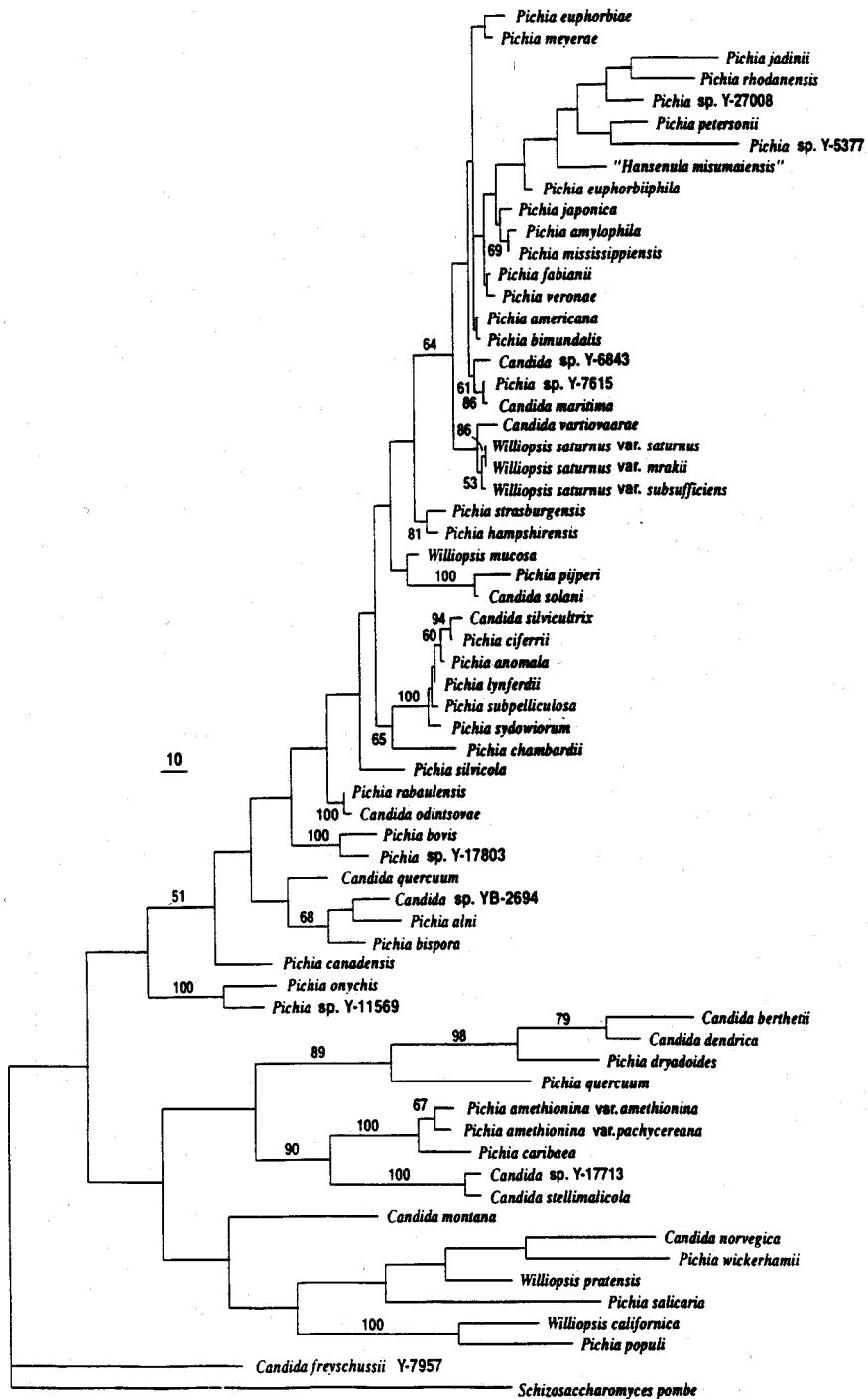


Figure 7. Phylogenetic tree of the *Pichia anomala* clade represented by 1 of 3 most parsimonious trees derived from maximum parsimony analysis. Tree length = 1616, CI = 0.333, RI = 0.624, RC = 0.208, HI = 0.667. Nitrate assimilating species such as *Pichia fabianii* and *P. bimundalis* are closely related to *P. mississippiensis* and *P. veronae*, which do not assimilate nitrate as a sole source of nitrogen.

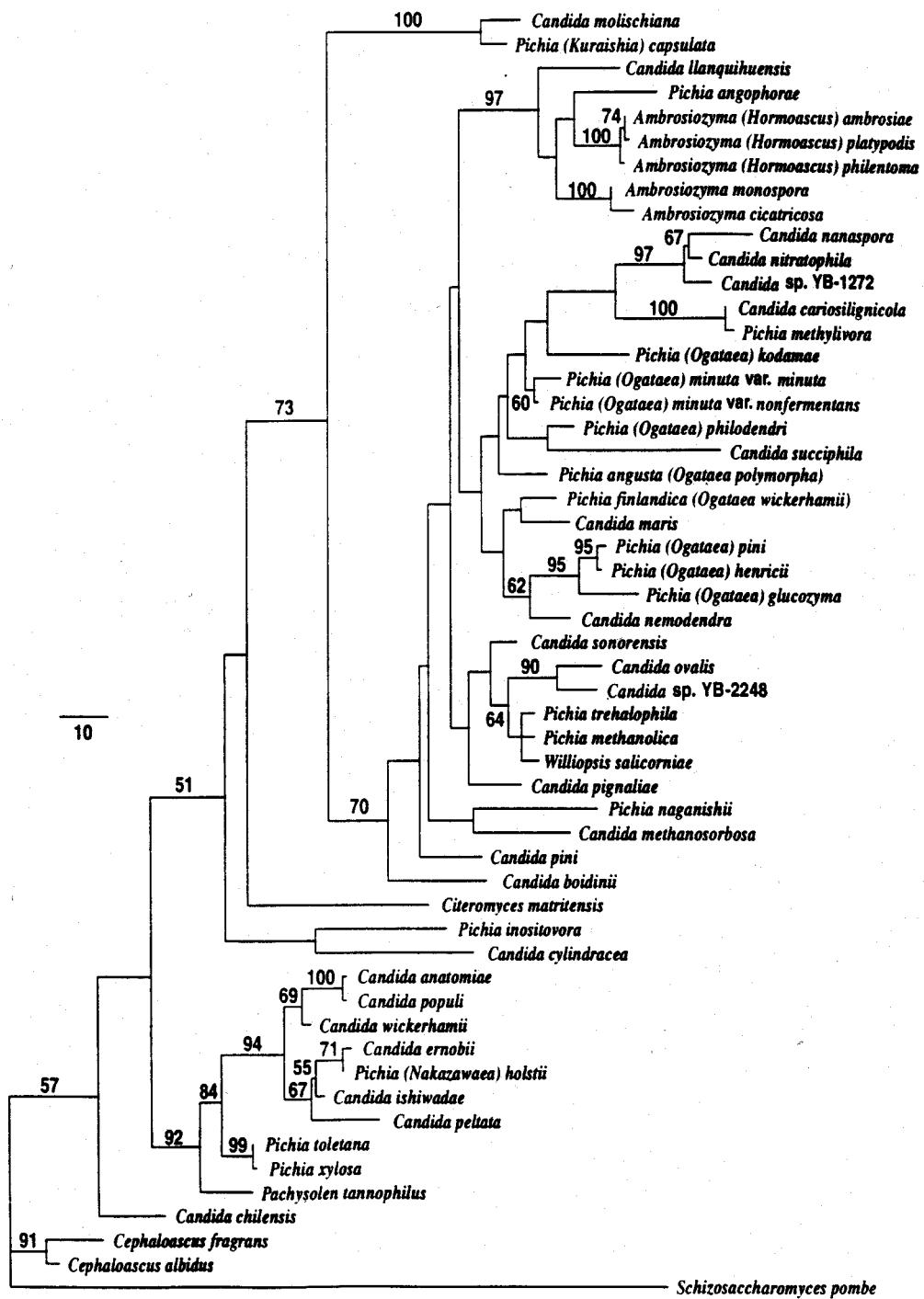


Figure 8. Phylogenetic tree of the methanol-assimilating yeasts and near relatives represented by 1 of 100 most parsimonious trees derived from maximum parsimony analysis. Tree length = 876, CI = 0.429, RI = 0.688, RC = 0.295, HI = 0.571. All members of the clade delimited by *Candida molischiana* and *C. boidinii* assimilate methanol except for *Williopsis salicorniae*, *Candida llanquihuensis*, *Pichia angophorae* and species of *Ambrosiozyma*. The other species shown do not assimilate methanol.

Phylogenetic analysis of domain D1/D2 (Figure 5) shows the family Lipomycetaceae to be monophyletic and statistically well-supported (bootstrap = 98%). However, the seven currently described teleomorphic genera, including the morphologically divergent *Dipodascopsis*, appear paraphyletic, suggesting that ascus morphology, ascospore ornamentation, and composition of coenzyme Q are unreliable predictors of kinship. Phylogenetic analyses of other gene sequences are needed to corroborate the present work, which suggests that all teleomorphic members of the Lipomycetaceae may belong in the single genus *Lipomyces* and that *Myxozyma* represents their anamorph.

Pichia, *Ambrosiozyma*, *Cephaloascus*, *Citeromyces*, *Dekkera*, *Issatchenka*, *Saturnispora*, *Williopsis*

The genus *Pichia*, which now includes species formerly placed in *Hansenula* (Kurtzman, 1984a), shows a broad range of phenotypic characters. Analysis of 18S rRNA sequences from a small number of species has confirmed the impression that *Pichia* is phylogenetically divergent and therefore artificial (Barns et al., 1991; Wilmotte et al., 1993). In the present analyses, species of *Pichia* are widely distributed among the ascomycetous yeasts (Figs. 6–10). Species closely related to *P. membranifaciens*, the type species of the genus, are shown in Figure 6. Among these taxa are members of *Issatchenka*, a genus characterized by roughened, spheroidal ascospores. Other members of the clade form hat-shaped ascospores, although some strains of *P. membranifaciens* are known occasionally to produce spheroidal spores. From the present analysis, *Issatchenka* appears to be a synonym of *Pichia*. Members of the *P. membranifaciens* clade are noted for assimilation of only a small number of carbon compounds, but a few species in other clades are similarly restricted.

Species assigned to *Saturnispora* form a small, distinct clade (Figure 6), as is also the case for species of *Dekkera* and its anamorph *Brettanomyces*. *Pichia delftensis* and *P. fluxuum*, despite their reported differences in ascospore morphology, are members of an isolated clade that is basal to *Dekkera* (Figure 6). The comparison shows *Candida vini* to represent the anamorph of *P. fluxuum* (Table 3).

Additional members of *Pichia* are shown in Figure 7. Several nitrate assimilating species formerly assigned to *Hansenula* are closely related to traditional non-nitrate assimilating *Pichia* species [e.g., *Pichia* (*Hansenula*) *fabianii* and *P. veronae*], which again

demonstrates that nitrate utilization is not of phylogenetic importance (Kurtzman, 1984a). Particularly noteworthy is the inclusion of most *Williopsis* species in this clade, which otherwise consists of species that form hat-shaped ascospores. A defining character of *Williopsis* is formation of saturn-shaped ascospores (Kurtzman, 1991). Yamada et al. (1994b) placed *Williopsis pratensis* in their newly described genus *Komagataella*. In the present study, *W. pratensis* clusters with *Williopsis californica* and *Pichia salicaria*, but bootstrap values are low.

Methanol assimilating yeasts, with the exception of *Pichia pastoris* (Figure 6), which Yamada et al. (1995a) transferred to *Komagataella*, appear closely related (Figure 8). Yamada et al. (1994a) and Mikata & Yamada (1995) proposed from differences in partial sequences of 18S and 26S rRNA, the transfer of some of these species to their newly described genus *Ogataea*. This transfer did not include *P. methylivora*, a member of the same clade in our analysis, or *P. trehalophila*, *P. methanolica*, *P. naganishii* or *Williopsis salicorniae*, which are basal to this clade. A more robust dataset is required to substantiate the present circumscription of *Ogataea* and the exclusion of the other methanol assimilating species noted, including the outlying *Pichia capsulata*, which was transferred to the genus *Kuraishia* by Yamada et al. (1994a).

Species of *Ambrosiozyma* and its synonym *Hormoascus* (Kurtzman & Robnett, 1995) form a distinct clade closely associated with the methanol assimilating yeasts but are characterized by hyphae with dolipore-like septa. *Pichia angophorae* and *Candida llanquihuensis* are members of this group but are not known to be hyphal.

The clade comprising *Pachysolen tannophilus*, *Pichia toletana*, *P. xylosa*, *P. holstii* and several species of *Candida* is statistically well-supported but phenotypically divergent. *Pachysolen* is unique to the group because it forms ascii at the ends of refractile tube-like ascophores. Yamada et al. (1994a) viewed *P. holstii* as an isolated species and transferred it to the new genus *Nakazawaea*.

Debaryomyces, *Lodderomyces*, *Wickerhamia*

Many of the species shown in Figure 9 were characterized earlier from an analysis of the D1/D2 rDNA domain (Kurtzman & Robnett 1997). Most notable is the clustering of *Candida albicans*, *C. viswanathii*, and several other clinically important yeasts with *Lod-*

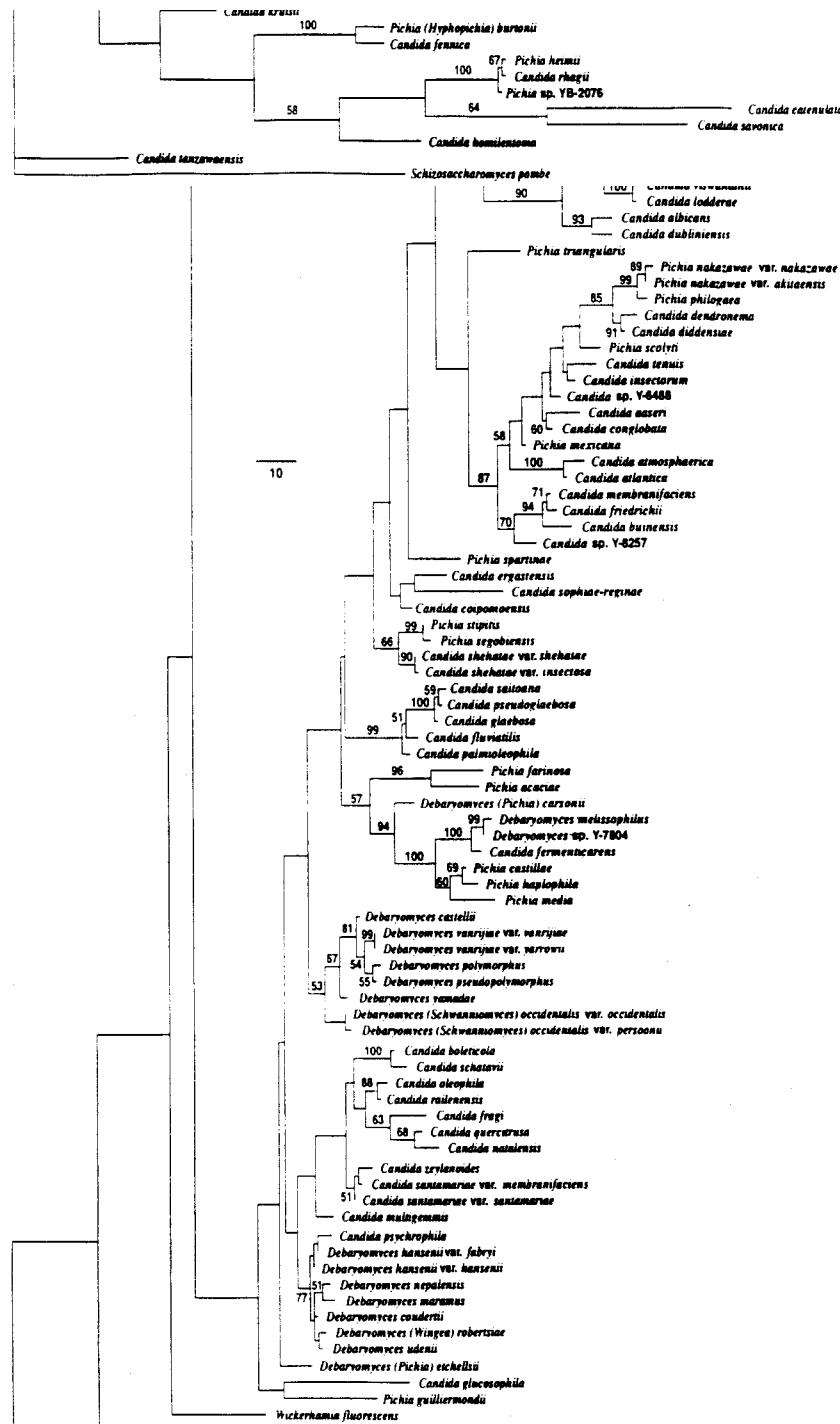


Figure 9. Phylogenetic tree of the *Debaryomyces/Lodderomyces* clade represented by 1 of 100 most parsimonious trees derived from maximum parsimony analysis. Tree length = 1433, CI = 0.355, RI = 0.678, RC = 0.240, HI = 0.645. *D. hansenii* is the type species of *Debaryomyces*.

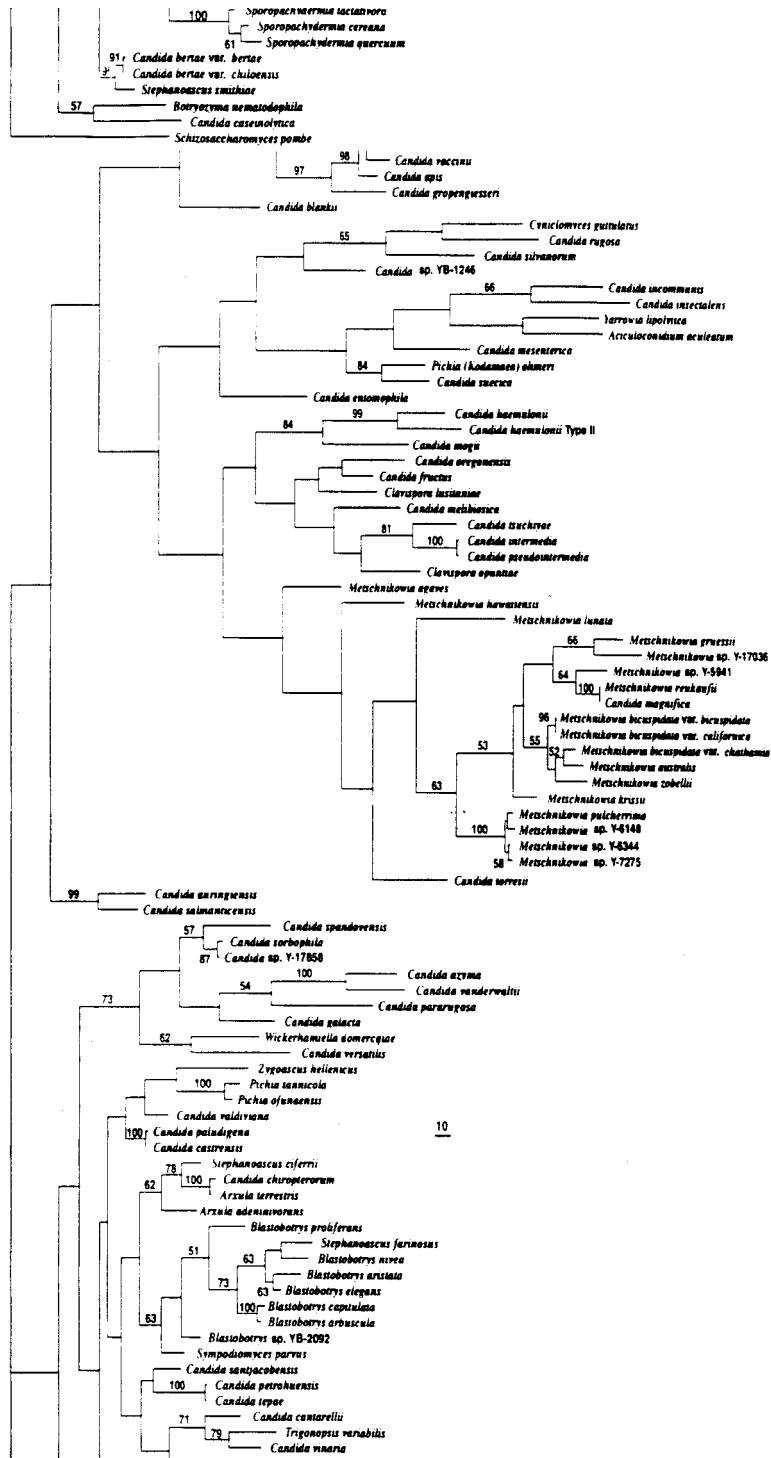


Figure 10. Phylogenetic tree of the *Stephanoascus/Metschnikowia* clade represented by 1 of 4 most parsimonious trees derived from maximum parsimony analysis. Tree length = 4137, CI = 0.240, RI = 0.592, RC = 0.142, HI = 0.760. *C. lusitaniae* is the type species of *Clavispora*; *M. bicuspis* is the type species of *Metschnikowia*.

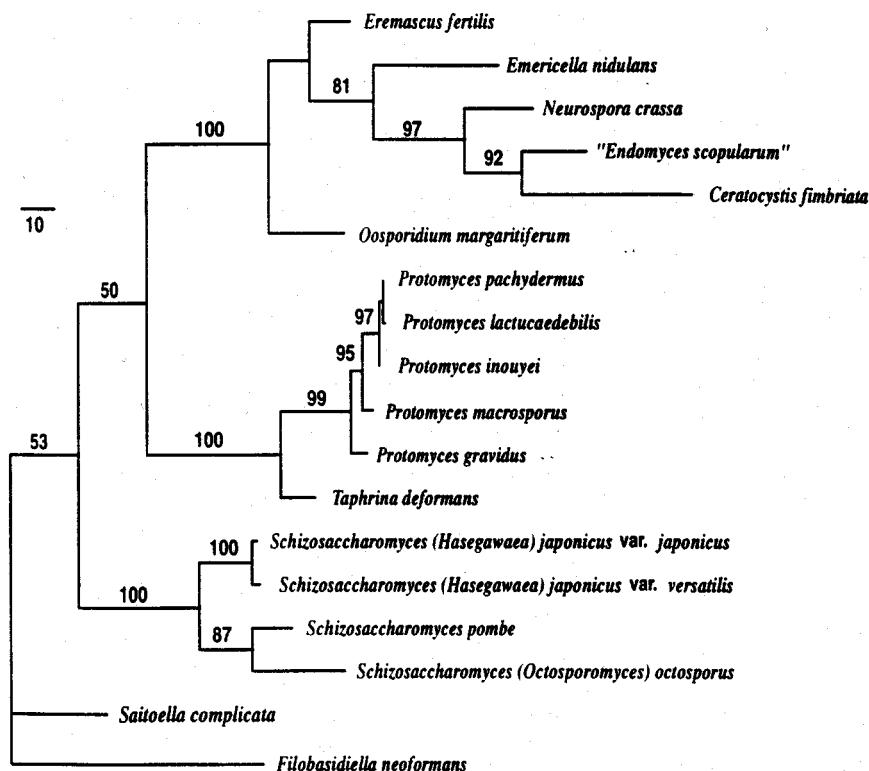


Figure 11. Phylogenetic tree of the "Archiascomycete" clade represented by the single most parsimonious tree derived from maximum parsimony analysis. Tree length = 748, CI = 0.623, RI = 0.722, RC = 0.450, HI = 0.377. The outgroup species in this analysis is *Filobasidiella neoformans*. See text for placement of *Saitoella complicata* in other analyses.

deromyces elongisporus. The present analysis places the newly described *C. sojae* near *C. tropicalis*.

Billon-Grand (1989) divided species of *Pichia* into three groups based on the type of coenzyme Q produced (Q-7, Q-8, Q-9) and transferred all Q-9 producing species that form hat-shaped ascospores to the newly described genus *Yamadazyma*. Our analysis places species assigned to *Yamadazyma* (e.g., *P. acaciae*, *P. guilliermondii* and *P. spartinae*) among several clades, all of which are characterized by coenzyme Q-9. *Pichia carsonii* and *P. etchellsii*, which also produce Q-9, were retained in *Pichia* by Billon-Grand (1989) because they form spheroidal ascospores. Yamada et al. (1992a,b) proposed the transfer of these two species to *Debaryomyces* on the basis of their comparison of partial 18S and 26S rRNA sequences. In the present analysis, *Debaryomyces* species separate into four clades that are represented by *D. hansenii*, *D. polymorphus*, *D. melissophilus* and *D. etchellsii*. *Debaryomyces carsonii* is a member of the *D. melissophilus* clade whereas *D. etchellsii* is basal to all other *Debaryomyces* clades. Basal branches of these four clades

are weakly supported and additional data are needed before generic boundaries can be confidently drawn. The clade containing *Pichia* (*Hypopichia*) *burtonii* is basal to the foregoing taxa and is characterized by species on long branches.

Clavispora, Cyniclomyces, Metschnikowia, Yarrowia

The genera *Metschnikowia*, *Clavispora*, *Yarrowia* and *Cyniclomyces* are weakly associated and characterized by highly divergent species (Figure 10). The divergence among *Metschnikowia* species was reported earlier from domain D2 analysis (Mendonça-Hagler et al., 1993), but inclusion of several undescribed species in the present study considerably shortened some terminal branches, which suggests that the genera in this group have numerous missing taxa. *Pichia ohmeri* also appears to be a member of this group but was transferred by Yamada et al. (1995b) to the genus *Kodamaea*.

Sporopachydermia, Stephanoascus, Wickerhamiella, Zygoascus

Stephanoascus comprises three highly divergent species and may be paraphyletic, whereas the three species assigned to *Sporopachydermia* are closely related (Figure 10). The monotypic genera *Wickerhamiella* and *Zygoascus* also appear to be associated with this clade. Other members of the clade include the morphologically varied anamorphic genera *Arxula*, *Blastobotrys*, *Sympodiomyces* and *Trigonopsis*, as well as various species of *Candida*. Further study of this group should help bring into perspective whether there is value in using seemingly unique morphological features for the definition of genera.

Schizosaccharomyces, Protomyces, Taphrina

Relationships among some of the 'Archiascomycetes' are shown in Figure 11. Yamada & Banno (1987) proposed assigning *Schizosaccharomyces octosporus* and *S. japonicus* to the genera *Octosporomyces* and *Hasegawaea*, respectively, because of differences in ascospore morphology, coenzyme Q composition and cellular linoleic acid content. We suggest retaining the three species in *Schizosaccharomyces* because branches are well-supported and the phylogenetic significance of the physiological differences is uncertain.

The genus *Protomyces* is poorly studied but may include up to 60 species (Reddy & Kramer, 1975), six of which are available from culture collections. *Protomyces inundatus* differs from *P. macrosporus* by one nucleotide and is considered to be a synonym of the former species. There is little divergence among three of the remaining five species suggesting that *P. pachydermus*, *P. lactucaeabilis* and *P. inouyei* may be conspecific. *Protomyces* has been assigned to the order Protomycetales whereas *Taphrina* is placed in the order Taphrinales. Our data suggest that both genera may be members of the Taphrinales, the order of taxonomic priority. *Saitoella complicata* is basal to *Schizosaccharomyces* in Figure 11, but when all ascomycetous yeast species from this study are included in the analysis, *S. complicata* is basal to *Taphrina*.

Conclusions

The work presented here, which compares extent of nDNA relatedness with nucleotide divergence in LSU domain D1/D2, indicates that nearly all currently recognized ascomycetous yeasts can be identified

from their unique D1/D2 sequences. Consequently, use of this database has the potential to markedly increase the accuracy of yeast identifications, and the effort required is less than that needed for preparation of the less reliable standard fermentation and assimilation tests. However, because several closely related species pairs, such as *Pichia stipitis/P. segobiensis*, show little or no divergence in domain D1/D2, identifications may need to be validated by nDNA reassociation.

The present study is the first to include essentially all known ascomycetous yeasts in the same molecular dataset. This has allowed the detection of numerous apparently synonymous species as well as recognition of previously unsuspected close relationships. Analysis of these data has given an overview of phylogenetic relationships among the ascomycetous yeasts. Basal lineages are not well resolved by this dataset, but the resolution may be comparable to that from 18S rDNA sequences. Consequently, additional gene sequences will need to be analyzed before most genera can be phylogenetically circumscribed.

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